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CELL BIOLOGY

Growth Regulation, Differentiation

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CELL AND SERUM DERIVED GROWTH EFFECTORS

1. EPIDERMAL GROWTH FACTOR INITIATES DNA SYNTHESIS AFTER A TIME-DEPENDENT SEQUENCE OF REGULATORY EVENTS IN SWISS 3T3 CELLS: INTERACTIONS WITH HORMONES AND GROWTH FACTORS.

Otto AM, Ulrich MO, Jimenez de Asua L
Friedrich Miescher-Inst., CH-4002, Basel, Switzerland
J Cell Physiol; 108(2):145-153 1981

Swiss mouse 3T3 cells in confluent and quiescent cultures were treated with epidermal growth factor (EGF) in various single and split doses and in combinations with insulin, hydrocortisone, prostaglandin E1 (PGE1), and prostaglandin F2 (PGF2). DNA synthesis was assayed using radioactive labeling. EGF stimulated the synthesis of DNA in quiescent cultures after a lag period of about 15 hr. The final rate of initiation of synthesis followed first-order kinetics and was quantified by a rate constant k. The value of k was affected by administrations of EGF during the prereplicative period. The minimum length of the lag phase was set by the first dose of

HE 20.3172/2

EGF, independent of concentration, while k was dependent on concen tration. Insulin alone did not stimulate DNA synthesis. In combination with EGF it showed a synergistic effect. Insulin did not effect the length of the lag phase. Hydrocortisone, when added within 8 hr of the addition of EGF or EGF plus insulin, inhibited the stimulatory effects of those substances. PGE1 inhibited DNA synthesis by increasing the lag phase. PGF2 had a synergistic effect in combination with EGF. The results suggest that a growth factor first induces a sequence of regulatory events leading to the initiation of DNA synthesis. Then it acts separately to regulate the rate of DNA synthesis. Prostaglandin and EGF apparently act on different regulatory events. (29 Refs)

2. A SERUM-FREE MEDIUM FOR A431 HUMAN EPIDERMOID CARCINOMA CELLS; INHIBITION OF GROWTH IN CULTURE BY MOUSE EPIDERMAL GROWTH FACTOR (MEETING ABSTRACT).

Barnes D
Department of Biological Sciences, University of Pittsburgh, Pittsburgh, PA, 15260
63rd Annual Meeting of the Endocrine Society, June 17-19, 1981, Cincinnati, Ohio. The Endocrine Society 385 pp., 1981.

We have developed a serum-free medium which supports A431 growth as well as serum containing medium (SCM). The basic nutrient medium (BNM) was a 1:1 mixture of Ham's F12 and Dulbecco-modified Eagle's medium containing 1.2g/l NaHCO3 and 15 mM 4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid. To BNM were added 4 factors stimulatory for A431 cell growth (BNM4F): 10 ug/ml insulin, 10 ug/ml transferrin, 5 ug/ml cold-insoluble globulin (CIg) and 0.5 mM ethanolamine. Preincubation of culture dishes with 5 ug/ml CIg or serum spreading factor could substitute for addition of CIg directly to medium in which cells were plated. Cells plated at a density of 2.5X10(3)/cm2 in BNM4F grew with a doubling time (TD) of 30 hr; cell number after 6 days was 85-100% of that in plates with SCM. A small, reproducible increase in cell number was seen if BNM4F was further supplemented with 2 ug/ml glycyl-histidyl-lysine; small, inconsistent increases were sometimes seen upon addition of 5 ng/ml parathyroid hormone or 100 ng/ml somatostatin. A431 cells also grew in BNM, but at a reduced rate. Addition of EGF at concentrations mitogenic for other cell types caused marked inhibition of A431 growth. Inclusion of EGF in the medium also reduced plating efficiency and prolonged the time lag before onset of growth after plating; cell number after 6 days in culture in BNM4F with 100 ng/ml EGF was about 10% of cell number in BNM4F. Inhibitory effects of EGF were detectable at 1 ng/ml in BNM4F and also were seen in BNM and SCM. Cells exposed to 10 ng/ml EGF in BNM4F for 72 hr and changed to BNM4F without EGF resumed growth with TD near that of unexposed cell after a lag of about 72 hr. Decrease in cell number due to inclusion of 10 ng/ml EGF in BNM4F could be prevented partially by the si multaneous addition of 2.5 ug/ml rabbit anti-EGF antibody. A431 cells produced tumors in athymic mice of either sex. (no Refs)

3. GLIA MATURATION FACTOR PROMOTES CONTACT INHIBITION IN CANCER CELLS.

Lim R, Nakagawa S, Arnason BG, Turriff DE
Brain Research Inst., Univ. Chicago, Chicago, IL, 60637
Proc Natl Acad Sci USA; 78(7,part2):4373-4377 1981

The effect of bovine glia maturation factor on the growth pattern of cancer cells was investigated in the rat glioma cell line 354A. When the cells were grown in the serum-free defined medium N2 in the absence of the factor, the cells proliferated with a doubling time of 24 hr without showing contact inhibition. After reaching confluency, the cell layer formed numerous foci from which heaps of cell colonies arose. The addition of glia maturation factor to the culture stimulated cell division in the logarithmic phase but prevented overgrowth once the cells arrived at confluency. The ability of glia maturation factor to restore contact inhibition suggests a regulatory role in normal and neoplastic cells. (Author abstract) (24 Refs)

4. BIOLOGICAL EFFECTS OF BOVINE GLIA MATURATION FACTOR ON GLIAL CELLS IN CULTURE.

Kato T, Yamakawa Y, Lim R, Turriff DE, Tanaka R
Dept. Biochemistry, Sch. Medicine, Nagoya City Univ., Mizuho-ku, Nagoya 467, Japan
Neurochem Res; 6(4):401-412 1981

The biological effects of bovine glia maturation factor (GMF) on glial cells are characterized with respect to cell age, DNA synthesis, and difference in responsiveness between glioblasts and astrocytoma cells. Rat glioblasts, which initially appeared as a continuous monolayer sheet, appeared multipolar, branched, and interconnected following exposure to GMF. C-6 astrocytoma cells also underwent morphological transformation upon addition of GMF, although none of the long processes typical of the glioblasts were observed. The morphological response to GMF was dependent on the age of the cell culture. An increase in DNA synthesis was observed in glioblasts following 12 hr of GMF stimulation. The max increase in DNA synthesis was observed 18 hr after stimulation. GMF stimulated cell proliferation in both glioblasts and C6 cell cultures. However, GMF increased the saturation density of glioblasts by twofold but did not change that of C6 cells. The incorporation of 3H-thymidine was inhibited at high GMF dose in C6 as observed in rat glioblasts. C6 cell required the same amount of GMF as in glioblasts to bring about a half-maximal thymidine incorporation: 400 nanog protein/ml of culture medium. The results indicated that normal cells, such as glioblasts may have a higher sensitivity to growth factors than tumor cells, such as C6. (10 Refs)

5. A SECOND MESSENGER REQUIRED FOR NERVE GROWTH FACTOR BIOLOGICAL ACTIVITY?

Heumann R, Schwab M, Thoenen H
Abteilung Neurochemie, Max-Planck-Institut fur Psychiatrie, D-8033 Martinsried, W. Germany
Nature (Lond); 292(5826):838-840 1981

The biological activity of mouse nerve growth factor (NGF) and NGF antibodies introduced directly into the cytoplasm of PC12 pheochromocytoma cells was studied by electron microscope autoradiography. The NGF and NGF antibodies were loaded onto guinea pig RBC ghosts that were then fused with the target cells in monolayer cultures. One ghost contained approx 44,000 NGF molecules or a quantity of antibodies sufficient to neutralize 26,000 NGF molecules. The direct introduction of NGF into the cytoplasm did not induce fiber outgrowth, which occurred only when NGF was added to the culture medium. Conversely, the injected NGF antibodies did not interfere with the fiber outgrowth induced by NGF in the culture medium. These results suggested that the biological effects of NGF are mediated by an interaction with specific cell-surface receptors and the generation of a second messenger, rather than by a direct action of intact NGF in the free cytoplasm or nucleus. (21 Refs)

6. LONG-TERM IN VITRO CULTURE OF MURINE MAST CELLS. II. PURIFICATION OF A MAST CELL GROWTH FACTOR AND ITS DISSOCIATION FROM TCGF.

Yung YP, Eger R, Tertian G, Moore MA
Dept. Developmental Hematopoiesis, Sloan-Kettering Inst. Cancer Res., New York, NY, 10021
J Immunol; 127(2):794-799 1981

Procedures are described for the isolation of a mast cell growth factor (MCGF) from medium conditioned by mitogen-activated splenic WBC (CM). Although optimal conditions for the production of MCGF in CM are identical to those for the production of T cell growth factor (TCFG), MCGF can be dissociated from TCGF after the first stage of purification on a DEAE-cellulose column. MCGF elutes from the column in the breakthrough fraction, whereas TCGF binds avidly to DEAE and is eluted only at high salt concentration. MCGF also differs from TCGF with respect to mol wt (as estimated by Sephadex G-150 chromatography) and sensitivity to trypsin. In addition, MCGF is produced by the murine myelomonocytic leukemia WEHI-3 and the radiation induced thymic lymphoma LBRM-33 cells, whereas TCGF is produced only by the latter in the presence of a mitogen. Another hemopoietically active factor, granulocyte colony-stimulating factor (G-CSF) present in media conditioned by WEHI-3 and LBRM-33 cells, however, shares a number of properties with MCGF. Although studies with purified or partially purified MCGF have thus far failed to reveal a correlation between MCGF and G-CSF, further biochemical analyses are necessary to dissociate MCGF from G-CSF. (Author abstract) (23 Refs)

7. THE CYTOSOL POLYPEPTIDES OF EHRLICH ASCITES TUMOR CELLS AND THEIR EFFECT ON DNA SYNTHESIS IN BALB C/3T3 CELLS.

Gabryel H, Bankowski E, Farbiszewski R
Dept. Inorganic Chemistry, Medical Sch., Bialystok 8, Poland
Biochem Biophys Res Commun; 101(1):153-157 1981

Polypeptides from Ehrlich ascites tumor cells were isolated and characterized in order to study the effect of these substances on the incorporation of 3H-thymidine into DNA of Balb c/3T3 mouse cells in culture. Sephadex gel filtration and chromatographic fractionation of tumor cell cytosol yielded five low mol wt polypeptides. They were homogeneous during electrophoresis and isoelectric focusing. Peptide I of mol wt 18,500 and isoelectric point 5.0 was the most abundant. It contained the carbohydrate components glucose, galactose, mannose, galactosamine, and N-acetylneuramic acid (respectively 150, 69, 161, 80, and 33 ug per mg alpha-amino nitrogen). The acidic peptide I was alone able to stimulate 3H-thymidine incorporation into Balb c/3T3 cells: it increased incorporation to 257.4% of control level. Peptides II-V, of basic nature and mol wt between 11,000 and 8,500, reduced incorporation to 36-68% of control. The growth-stimulating glycopeptide isolated here illustrates the role polypeptides from tumor cells may play in the regulation of cell growth. (14 Refs)

8. KIRSTEN MSV TRANSFORMED CELLS GROW IN SERUM FREE/MITOGEN FREE MEDIUM (MEETING ABSTRACT).

Kaplan PL, Ozanne B
University of Texas Health Science Center at Dallas, Dallas, TX, 75235
RNA Tumor Viruses, May 20-24, 1981, Cold Spring Harbor, New York. Cold Spring Harbor Laboratory 294 pp., 1981.

Kirsten mouse sarcoma virus (MSV) transformed rat and mouse fibroblasts were grown in a defined serum free medium to which no exogenous mitogens were added. The minimally required additions for continual growth were transferrin for Kirsten MSV transformed normal rat kidney (NRK) cells, and transferrin and insulin for other Kirsten MSV transformed cell lines including other isolates of KNRK. Under these conditions, classical growth factors, such as epidermal growth factor, fibroblast growth factor, and PDGF, which are potent mitogens for many cell types, had no effect on cell growth. Cells transformed with the temperature sensitive mutant of Kirsten MSV grew in this medium only at the permissive temperature for transformation however, at the nonpermissive temperature growth occurred if crude conditioned medium from the transformed cells was added. Like the temperature sensitive Kirsten MSV transformed Rat-1 cells, normal Rat-1 and NRK cells did not grow in this medium. Unlike the temperature sensitive transformed cells though, crude conditioned medium did not induce these normal cells to grow. These data suggest that while ectopically produced growth factors are associated with the growth of Kirsten MSV transformed cells, alone they are not sufficient to induce the growth of normal cells in an otherwise mitogen free medium. (no Refs)

9. CLONAL ANALYSIS OF THE RESPONSE OF HUMAN MYELOID LEUKEMIC CELL LINES TO COLONY-STIMULATING ACTIVITY.

Ruscetti FW, Collins SJ, Woods AM, Gallo RC
(c/o Gallo) Lab. Tumor Cell Biology, NCI, Bethesda, MD, 20205
Blood; 58(2):285-292 1981

The recent development of two continuously proliferating human myeloid leukemic cell lines (HL-60 and KG-1) that respond to CSA provides an opportunity for a detailed study of the interaction of CSA with leukemic myeloid cells. Here we report on the colony-forming ability of HL-60 and KG-1 over an extended culture life of the cells. Several different sources of human CSA of different stages of purity enhanced colony formation of these cells. CSA, obtained from conditioned media from an SV-40 transformed human trophoblast, was partially purified, and its activity for normal bone marrow copurified with the activity that stimulated HL-60 colony formation. Over 100 clones of HL-60 were developed and tested for their response to CSA. All responded to CSA by showing an increase in colony size and number. However, none of the colonies formed from any of the 100 clones differentiated in response to CSA despite the fact that many chemicals can induce differentiation of HL-60. Since HL-60 forms spontaneous colonies without the addition of any exogenous stimulating factors, HL-60 conditioned media and cell extracts were tested for the production by these cells of their own endogenous growth-promoting activity (such as a CSA-like molecule). No growth-promoting endogenous activity was found that stimulated normal bone marrow or HL-60 colony formation even after concentration and fractionation methods were employed. These experiments suggest that (1) the effect of CSA markedly favors proliferation over differentiation in these cell lines; (2) CSA is unlikely to suppress growth of the type of leukemic myeloid cells that HL-60 and KG-1 represent; and (3) if HL-60 cells produce their own growth-promoting factor it is not detectable in the media. (Author abstract) (29 Refs)

10. STIMULATION AND SUPPORT OF HAEMOPOIETIC STEM CELL PROLIFERATION BY IRRADIATED STROMA CELL COLONIES IN BONE MARROW CELL CULTURE IN VITRO.

Mori KJ, Izumi H, Seto A
Dept. Microbiology, Faculty Medicine, Kyoto Univ., Kyoto 606, Japan
J Radiat Res (Tokyo); 22(1):109-115 1981

The ability of stromal cell colonies in culture to stimulate stem cell proliferation and to support the increased production of stem cells in vitro was studied. Culture of bone marrow cells from male DDY mice resulted in the formation of adherent cell colonies within 3 wk. These colonies were irradiated in vitro and normal bone marrow cells were reinoculated on top of the irradiated stromal cell colonies. Mostly insignificant decreases were observed in the number of cells in suspension following inoculation on the irradiated adherent cell colonies compared to the number in the non-irradiated control culture. The number of pluripotent stem cells (CFUs) per flask decreased according to the dose of xrays applied to the adherent cell colonies when assayed 1 wk after irradiation. This seemed to indicate that the hemopoietic supportive activity of the stromal cells was injured during the short period after irradiation. The reduction of the CFUs was, however, slight and a significant increase was observed in the stem cell number thereafter. Similar but less apparent effects were observed on the number of granulocyte-macrophage progenitor cells (CFUc). To illustrate the results more clearly, the number of CFUs per flask and that of CFUc was plotted as percentage control against the time after irradiation. The results indicated that the stromal cells produce factors which stimulate stem cell proliferation. (19 Refs)

11. STUDIES OF LYMPHOCYTE PROLIFERATIVE RESPONSE TO AUTOLOGOUS TUMOR EXTRACT IN VARIOUS CANCER PATIENTS.

Honjo H
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Nichidai Igaku Zasshi; 40(2):129-139 1981

Lymphocyte proliferative responses to the autologous tumor extract (LP responses) were examined in 53 patients with various cancers. Preoperatively, positive LP responses stimulation index (SI) of 2.00 or greater were observed in 19 patients. Analysis of the positive responders according to the histological type of the tumor revealed that patients with neuroblastoma gave the most frequent positive responses, followed in order by colorectal, stomach and breast cancer patients. Analysis of the relationship between the frequency of positive responses and extent of disease revealed that patients with intermediate disease gave the most frequent positive responses, followed in order by those with widespread and localized disease. No statistically significant relationship was observed between the LP responses and prognosis of the disease or lymphocyte proliferative responses to phytohemagglutinin. Serial determinations of the LP responses in adult cancer patients revealed that the SI values of patients who had curative surgery fell to low levels at about 6 mo after the operation and remained low until recurrence developed. On the other hand, in patients who had noncurative surgery, SI values were highest preoperatively, decreased after surgery and remained low. Serial determinations of the LP responses in children with cancer revealed that the SI values fell to low levels after complete or partial tumor removal and remained low until recurrence or tumor regrowth was detected clinically. (25 Refs) (In JPN)

12. ASSAY OF AN ACTIVITY IN THE SERUM OF PATIENTS WITH DISORDERS OF THROMBOPOIESIS THAT STIMULATES FORMATION OF MEGAKARYOCYTIC COLONIES.

Hoffman R, Mazur E, Bruno E, Floyd V
Dept. Internal Medicine, Yale Univ. Sch. Medicine, Hematology Section, LCI 812, 333 Cedar St., New Haven, CT, 06510
N Engl J Med; 305(10):533-538 1981

An assay system for committed stem cells or human megakaryocytic colony-forming units (CFU-M) in which an antiserum directed against platelet glycoproteins (PGPs), in concert with indirect immunofluorescence staining, was used to identify megakaryocytic colonies grown in vitro. A mean 12.4 colonies/5 x 10(5) cells cultured were grown from marrow mononuclear cells of 10 hematologically normal subjects. Serum samples from 11 patients with thrombocytopenia associated with reduced megakaryocyte numbers, when substituted for 10% of normal serum in the final culture vol, enhanced CFU-M-derived colony formation by 200-1,840%. Eight serum samples from subjects with equally severe thrombocytopenia but normal or elevated marrow megakaryocytes did not alter CFU-M-derived colony formation; nor did serum specimens from 11 persons with thrombocytosis or from 3 aplastic anemia patients. The cloning of megakaryocyte colonies increased linearly with increasing concentration of serum from the thrombocytopenia patients with diminished numbers of marrow megakaryocytes, throughout the tested concentration range of 5-30%. Serum concentrations greater than 10% did not affect formation of CFU-M-derived colonies in the other patient groups. The megakaryocytic-colony-stimulating activity (Meg-CSA) seemed to be inversely related to numbers of marrow megakaryocytes. Meg-CSA appears to act at the level of the CFU-M; variations in Meg-CSA levels detected in different disease states suggest that alterations in production of this stem-cell regulator have physiologic importance. (31 Refs)

13. TUMOR-INDUCED SUPPRESSION OF MARROW STROMAL COLONIES.

DeGowin RL, Gibson DP, Knapp SA, Wathen LM
Dept. Medicine, Univ. Iowa Hosp., Iowa City, IA, 52242
Exp Hematol; 9(7):811-819 1981

The studies reported in this paper evaluated one of the mechanisms by which extramedullary tumor caused anemia, neutrophilia, medullary erythroblastopenia, and suppression of marrow stromal cells (MSC) in tumor bearing mice. Since MSC have been shown to support hemopoiesis, we asked whether the tumor released a suppressor which directly inhibited MSC colonies, and if it did, whether or not it was prostaglandin E (PGE). We found that co-culture of Ehrlich ascites carcinoma (EAC) and sarcoma (S-180) cells with normal mouse bone marrow cells profoundly suppressed formation of MSC colonies. At concentrations exceeding 15% of the medium, tumor-conditioned media not only suppressed MSC colonies but also enhanced the growth of granulocyte-macrophage colonies (CFUC) in vitro like colony stimulating factor (CSF) from other sources. It did not suppress CFUE, nor did it inhibit growth or kill cells other than MSC in bone marrow cultures. Fibroblasts grew luxuriantly in 20% conditioned media from Ehrlich ascites carcinoma cells. Concentrations of PGE2 required to suppress MSC colonies greatly exceeded those detected in media conditioned by S-180. Production of PGE by S-180 cells was inhibited by growing the tumor cells in the presence of indomethacin, but the supernatant media, devoid of PGE, still markedly suppressed the growth of MSC from normal marrow. Because the tumor produced CSF, we

tested the suppressive effect of CSF in post-endotoxin serum to find that concentrations of 10 to 15% inhibited MSC colony growth. The results show that these tumors produced a substance, possibly CSF, that selectively inhibited MSC colony growth in vitro. It is conceivable that suppression of the supportive tissue (MSC) for erythropoiesis in the bone marrow by tumor led to diminished erythroblasts in that site. (Author abstract) (18 Refs)

14. REGULATION OF ANTIGEN-INDUCED LYMPHOPROLIFERATION BY BSA GRADIENT-SEPARATED T CELL FRACTIONS.

Emmons RP, Carvalho EM, Horwitz DA
Div. Rheumatology, Dept. Internal Medicine, Univ. Virginia, Box 412, Charlottesville, VA, 22908
J Immunol; 127(3):821-824 1981

Human T cells from six volunteers immunized with key-hole limpet hemocyanin (KLH) or tetanus toxoid were fractionated on bovine serum albumin (BSA) gradients. These T cell fractions were then recombined with autologous unseparated mononuclear cells or unseparated T cells to determine the effect of each fraction on the proliferative (DNA synthetic) response to the immunizing antigen. Using this separation technique, we were able to define two fractions of T cells of widely different densities that had a suppressive effect on the proliferative response to KLH of unfractionated T cells. The demonstration of suppressive activity was possible only if cells were tested within 8 wk of immunization with KLH. These suppressive T cell fractions differed not only in density but also in surface characteristics. Fraction 1 cells had a high proportion of Ia+ cells but no Fc receptors for IgG (FcRG-) were seen, whereas cells from Fraction 4 (of higher density) were Ia- and were composed of 12.1 +-1.2% FcRG+ cells. This suppression was shown to be specific for the immunizing antigen. One individual who had been immunized with KLH for more than 1 yr in which suppression of KLH-induced proliferation was no longer demonstrable was given primary immunization to tetanus toxoid. Suppression could be demonstrated in Fractions 1 and 4 specific for tetanus toxoid; there was no demonstrable effect of these T cell fractions on the response to KLH. We postulate that these are different functional populations of suppressor T cells that regulate antigen specific lymphoproliferation. (Author abstract) (26 Refs)

15. MECHANISMS CONTROLLING THE KINETICS IN PROLIFERATION AND DIFFERENTIATION OF POPU LATIONS OF MOUSE MYELOID LEUKEMIC CELLS IN VITRO.

Hayashi M, Okabe-Kado J, Hozumi M
Dept. Chemotherapy, Saitama Cancer Center Res. Inst., Saitama 362, Japan
J Cell Physiol; 108(2):123-134 1981

Cells from 2 clones, B24 and DR-3, of mouse myeloid leukemic cell line M1 were grown in a conditioned (CM) medium prepared from Syrian hamster embryo cells. Differentiation of the clone cells was measured at different times and at different concentrations of CM. Differentiation was measured by the ratio of nuclear area to cell area (NCR) and by autoradiographic labeling methods. During cell differentiation the NCR declined from 55% to 10%. Among treated cells those with a high NCR were non-adherent. Those with a low NCR were adherent and had become phagocytic. When cells were grown on CM, and then the CM was removed, some of the cells showed a raised NCR. This suggests that cells which have passed the boundary of about 40-35% are competent to differentiate without CM, while those which have not yet reached this boundary are not. The results suggest that (1) increasing the concentration of CM enhanced the differentiation of the cells and decreases differentiation transit time, (2) the activities of DNA synthesis in individual cells decreased at the stage of cell differentiation at which the NCR was between 30 and 50%, and this decrease was independent of culture time and CM concentration. A stochastic model for the kinetics of proliferation and differentiation of cells simulated the reported observations on the production of differentiated cells. (30 Refs)

16. APPEARANCE OF ASIALO GM1 GLYCOSPHINGOLIPID ON THE CELL SURFACE DURING LYMPHOKINE-INDUCED DIFFERENTIATION OF M1 CELLS.

Akagawa KS, Momoi T, Nagai Y, Tokunaga T
Dept. Tuberculosis, Natl. Inst. Health, Shinagawa-ku, Tokyo 141, Japan
FEBS Lett; 130(1):80-84 1981

The effect of lymphocyte products on the differentiation of M1 cells (M1 cell line was established from a myeloid leukemia of SL mice) was studied. Lymphokine (LK)-rich sera was obtained by immunization of ICR mice with viable BCG cells and old tuberculin (OT). Concanavalin A (con A)-induced LK were obtained by stimulating normal mouse spleen cells with con A-agarose. LK induced in vivo with OT was shown to induce the differentiation of M1 cells into macrophages. To test whether the differentiation-inducing factor contained in LK was different from macrophage-activating factor (MAF), con A-LK were incubated with M1 cells. MAF activity of LK after absorption with M1 did not differ from that of unabsorbed LK. Indirect immunofluorescence of M1 cells and thin-layer chromatography of their glycosphingolipids showed that LK-induced differentiation was accompanied by the appearance of asialo GM1 on the surface of M1 cells. (25 Refs)

17. LIVER PLASMA MEMBRANES AND PROTEOGLYCAN PREPARED THEREFROM INHIBIT THE GROWTH OF HEPATOMA CELLS IN VITRO.

Kawakami H, Terayama H
Zoological Inst., Faculty Science, Univ. Tokyo, Hongo, Bunkyo-ku, Tokyo 113, Japan
Biochim Biophys Acta; 646(1):161-168 1981

Plasma membranes from rat liver or kidney inhibited the growth of hepatoma (AH-130) cells in vitro. AH-130 plasma membranes or RBC ghosts inhibited the growth of AH-130 cells less effectively. The inhibitory activity of liver plasma membranes was lost by heat treatment, or mild protease (papain or bromelin, but not trypsin or pronase) treatment, whereas it was retained after sialidase treatment or delipidation by ethanol/ether. Proteoglycan (proteoheparin sulfate) prepared from liver plasma membranes inhibited the growth of AH-130 cells, but heparin sulfate was less active. The inhibitory activity of liver plasma membranes seemed, however, not to be ascribable solely to proteoheparin sulfate associated with plasma membranes. Preliminary investigations suggested that the mol wt 40,000 component may be a major inhibitory principle in liver plasma membranes. (Author abstract) (18 Refs)

18. CONDITIONS REQUIRED FOR THE INHIBITION OF IN VITRO GROWTH OF A MOUSE MYELOMA CELL LINE BY ADHERENT BONE-MARROW CELLS.

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Dept. Cell Biology, Weizmann Inst. Science, P.O. Box 26, 76100 Rehovot, Israel
Cell Tissue Kinet; 14(5):479-488 1981

The in vitro growth of the MPC-11 myeloma cell line was inhibited when these cells were co-cultured with adherent cells from mouse bone marrow. This growth inhibition involved prolongation of the specific population doubling time of the MPC-11 cell line. Control cultures of MPC-11 cells exhibited an av doubling time of 14-15 hr, whereas in the presence of adherent layers the length of the doubling time was up to 28 hr. This prolongation in the doubling time did not depend on the duration of incubation, but on the relative proportions of tumor cells and adherent cells employed. MPC-11 cells seeded in relatively high starting cell concentrations partially overcame the growth inhibition. The inhibitory activity of adherent cells from the bone marrow did not appear to be due to production of soluble factor(s), since media conditioned by adherent cells did not affect cell growth. Moreover, in modified cocultures in which MPC-11 cells grew physically separated from the adherent layers, only marginal growth inhibition activity was observed. The possibility that cell-to-cell interactions lead to the inhibition of growth of MPC-11 cells by adherent cells from the bone marrow, and the implications of these findings to the control of cell growth by the hemopoietic microenvironment, are discussed. (Author abstract) (13 Refs)

19. INHIBITION OF SIMIAN VIRUS 40 GROWTH IN A KIDNEY CELL LINE BY AN ENDOGENOUS INHIBITOR OF RENAL CELL PROLIFERATION.

Campadelli-Fiume G, Sinibaldi P, Busi C, Mattioli A, Fiume L
Istituto di Microbiologia e Virologia, Univ. Bologna, Via S. Giacomo 12, 40126 Bologna, Italy
Ann Sclavo; 23(1):52-56 1981

Chalones are non-cytotoxic, tissue-specific, non-species-specific substances that inhibit growth apparently by hindering deoxyribonucleotide polymerization. The effect of an inhibitor of epithelial kidney cell proliferation (IKCP) on viral proliferation was tested. BSC1 cells, a line of epithelial-like cells originated from African green monkey kidney, were infected with simian virus 40 (SV40). After virus adsorption IKCP was added to the cultures. All IKCP preparations hindered BSC1 cell proliferation. Above a threshold dose a dose-response relationship was observed. The IKCP preparations inhibited SV40 replication at the same concentrations which affected cell growth and which were non-cytotoxic. Apparently a number of endogenous cell growth inhibitors exhibit antiviral activity. Since they are non-cytotoxic, they may have pharmacological implications. (18 Refs)

20. GRANULOCYTIC CHALONE (GC) EFFECT ON THE GROWTH PATTERN OF CONTINUOUS LEUKAEMIC CELL LINES.

Foa P, Maiolo AT, Lombardi L, Binda U, Polli EE
Istituto di Clinica Medica I, Univ. Milan, Milan, Italy
Cell Biol Int Rep; 5(8):761 1981

In vitro actions of the granulocytic chalone (GC) on the growth pattern of leukemic cell populations were assessed. GC was prepared by incubating peripheral ox WBC in saline (4 hr, 37 C) and purifying the conditioned medium by ultrafiltration on Amicon membranes and the fraction with a nominal mol wt = 500-10,000 dalton was further purified by Sephadex G25 filtration. 100 ug/ml GC were added every 12 hr for 48 hr to the following continuous leukemic cell lines growing in suspension culture: rat acute myeloid W25, human acute promyelocytic HL60, and human T lymphoblastic line 8402. Cell proliferation, assessed by serial count, was significantly inhibited in both the rat W25 and human HL60 cultures, but not in the human 8402 cells (respective doubling times in hr, versus untreated controls, were 21.0/15.0, 52.5/36.0, and 29.5/29.5). GC-induced inhibition of growth in W25 and HL60 cell lines was spontaneously reversible approx 18 hr after discontinuing treatment, when growth rates returned to control levels. GC in vitro was shown to exert a powerful but non-toxic, myeloid-but not species-specific, inhibitory action on leukemic cell populations. (4 Refs)

21. HOG KIDNEY DIAMINE OXIDASE CONVERSION OF BIOGENIC DIAMINES TO INHIBITORS OF CELL PROLIFERATION.

Gaugas JM, Dewey DL
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J Pathol; 134(3):243-252 1981

Whether diamine oxidation by purified hog kidney diamine oxidase (DAO) adversely affects the proliferation of cultured mammalian cells was investigated. Arrest of cell proliferation followed the addition of 0.05 Unit/cc DAO together with 1,3-diaminopropane, putrescine, and cadaverine, but not histamine, to cell cultures; doses required to inhibit Bri8 lymphocyte proliferation by 50% (ID50) were respectively 125, 60, and 28 uM, and 128 milliM. Bri8 lymphocytes, V79 fibroblasts, and HP2 melanoma cells were approx equally susceptible to the system. Byproducts of DAO-substrate interaction, H2O2 and NH4+, are themselves cytotoxic but were apparently not responsible for antiproliferation in this experimental system: addition of 300 Unit/cc catalase failed to prevent arrest of proliferation and the ID50 for added NH4+ was 125 uM, a quantity too high to account for cadaverine and putrescine oxidation cytotoxicity. The known product of putrescine-DAO reaction, 4-aminobutyraldehyde (ABA), is compatible with a radio-labeled compound separated from the reaction mixture by ion-exchange chromatography. Supporting data included measurements of labeled putrescine, oxidation rate, level of primary product (ABA), and ABA lability (half-life approx 2 hr). An estimated ID50 for ABA was 21 uM. The circumstantial evidence suggested that the amine aldehyde products of diamine oxidation have a potent antiproliferative effect on cells. (15 Refs)

HORMONES/ STEROIDS/ PROSTAGLANDINS AS GROWTH EFFECTORS

22. THE REGULATION OF PROGESTERONE RECEPTOR BY 17BETA ESTRADIOL AND TAMOXIFEN IN THE ZR-75-1 HUMAN BREAST CANCER CELL LINE IN DEFINED MEDIUM.

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Medicine Branch, Clinical Oncology Program, Div. Cancer Treatment, NCI, NIH, Bethesda, MD, 20205
J Receptor Res; 2(1):17-27 1981

The effects of 17beta estradiol, tamoxifen, and insulin on progesterone receptor concentration and cell growth in the ZR-75-1 human breast cancer cell line were examined. Cells derived from a malignant ascitic effusion in an infiltrating duct carcinoma were grown for up to 12 days in defined serum-free medium supplemented by hormones. Progesterone receptors were assayed by radioimmunoassay with 3H-R5020. Estradiol was optimal as a stimulator of progesterone receptor activity at physiologic concentrations (10(-8) and 10(-10) M). Removal of 10(-8) M estradiol led to cessation of cell growth and undetectable receptor concentration after 6 days. Readdition led to exponential growth and recovery of normal receptor activity (up to 150 femtomoles/10(6) cells) after 3 days. Presence of 5 x 10(-7) M insulin did not affect receptor concentration, although cell growth depended upon it. No concentration of tamoxifen tested (10(-6), 10(-7), or 10(-8) M) showed an ability to stimulate receptor activity. In this cell line the presence of progesterone receptor activity is not directly coupled with cell proliferation. The sensitive regulation of progesterone receptor by estrogen continues during alterations in growth secondary to insulin . (18 Refs)

23. STEROID RESPONSIVENESS OF THE HUMAN CELL LINE NHIK 3025.

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Inst. Marine Biochemistry, N-7034 Trondheim-NTH, Norway
Acta Endocrinol (Kbh); 97(4):551-558 1981

The human cell line NHIK 3025, derived from a carcinoma of the uterine cervix, contains a glucocorticoid and an androgen receptor. The effect of various natural and synthetic steroid hormones and antihormones on growth rate of these cells was therefore investigated. Cells grown in Eagle's MEM with 10% fetal calf serum exhibited reduced growth when cultured with dexamethasone due to prolongation of the cell cycle. Glucocorticoid anti-inducers like progesterone had no significant effect on cell growth. Methyltrienolone (R 1881) or 5alpha-dihydrotestosterone did not affect cell proliferation. The reported shortening of the cell cycle by testosterone is probably not directly connected with activation of the androgen receptor present, but possibly dependent on metabolic conversion of testosterone to the more potent growth stimulator 4-androstene-3beta,17beta-diol. The effect of several anti-androgens was also studied. The non-steroidal anti-androgens flutamide and SCH 16483 had no significant effect on cell proliferation. It was, however, found that a number of steroid anti-androgens, including R 2956, stimulated cell growth. A significant stimulatory effect by R 2956 was seen within the first cell generation, 4-androstene-3beta,17beta-diol had to be present during 2 days, and testosterone for even longer times before a similar effect on cell growth could be obtained. (Author abstract) (24 Refs)

24. ESTROGEN AND PROLACTIN EFFECTS ON CELL GROWTH AND PRECURSOR INCORPORATION OF A HUMAN MAMMARY CARCINOMA CELL LINE (CAMA-1) (MEETING ABSTRACT).

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63rd Annual Meeting of the Endocrine Society, June 17-19, 1981, Cincinnati, Ohio. The Endocrine Society 385 pp., 1981.

CAMA-1 cells are maintained in the log phase for 7 days in Eagle's minimum essential medium with Earle's Salts, supplemented with 10% fetal bovine serum (FBS) without further feeding. The growth rate of cells (by Coulter counter) increases proportionally according to the amount of serum added (0-25%); and further increase can be facilitated by estrogen (1 to 10 nM) added either to FBS or dextran-charcoal treated FBS (DCFBS). These cells cease dividing by the 2nd passage in DCFBS. Cells grown in DCDBS with 1 nM estradiol (E2) resume partial division. Addition of 1 nM E2 to cells grown in DCFBS without E2 for 3 passages causes a 2-fold increase in cell yield. CAMA-1 cells propagate better in a 10% serum substitute (SS) with 1 nM E2 and 1% FBS than in 25% FBS. However, estrogen induces a smaller increase in cell gro wth in the former condition than in the latter. Estrogen stimulates a dose-dependent increase of thymidine, uridine and leucine uptake into cells; the stimulation is not observed in SS, unless 1% FBS or DCFBS is added. Ovine prolactin has no effect on cell growth when added alone or with E2 to cultures supplemented with DCFBS or SS, but can augment estrogen-induced uptake of precursors in DCFBS and partially restores estrogen-induced precursor uptake in SS. These results show that a) CAMA-1 cells are estrogen-dependent, and also respond to prolactin in culture, b) estrogenic effect on cell growth in culture may require serum factors, and c) synergism of estrogen and prolactin in cellular events occurs in human breast cancer cells in culture. (no Refs)

25. THE ROLE OF ESTROGEN AND PROLACTIN IN GROWTH AND REGRESSION OF RAT MAMMARY TUMOR MTW9 (MEETING ABSTRACT).

Hollander VP, Volchek A, Feldman M, Diamond EJ
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4th Annual San Antonio Breast Cancer Symposium, Nov 6-7, 1981, San Antonio, Texas Cancer Therapy and Research Center of South Texas; 1981

MTW9 as maintained in our laboratory will not grow unless serum prolactin of implanted hosts is increased to approx 500 ng/ml by administration of dopamine antagonists like perphenazine; the resulting tumor, MTW9-P, always regresses after ovariectomy whether or not serum prolactin is maintained at high levels by continued drug treatment. When serum prolactin is maintained at 5-50ug/ml by coimplantation with the mammosomatotropic tumor MtTW10, the resulting mammary tumor does not show ovariectomy-induced regression (OIR). Administration of prolactin, growth hormone or ACTH alone, or in combination, fails to prevent OIR in MTW9-P, but transplantation of MtTW10 to such hosts does prevent OIR. A number of other treatments prevent OIR: 5mg/d progesterone, testosterone, flutamide, norethindrone; 1 and 10ug/wk estradiol valerate (EV). Prevention of OIR by progesterone and low dose EV does not increase serum prolactin. At these doses MTW9 shows little growth. Cortisol, 17-hydroxyprogesterone caproate, 5, 10 mg/d; EV, 10ug/d; fail to prevent OIR. After OIR in MTW9-P, treatment with 1-10ug/wk EV, 5mg/d progesterone, 5mg/d testosterone cause regrowth of nodules.

Since steroids do not stimulate growth of dimethylbenzanthracene tumors in hypophysectomized hosts, these data are not consistent with the hypothesis that prolactin is the primary growth factor for this mammary tumor nor with a secondary role for estrogen as a stimulus for hyperprolactinemia. They do suggest that MtTW10 secretes a tropic substance other than prolactin and that several steroidal activities increase this secretion. (no Refs)

26. STUDIES ON THE EFFECTS AND MECHANISM ON ACTION OF ESTRADIOL-17 BETA AND MEDROXYPROGESTERONE ACETATE UPON THE MTTW15 MAMMOSOMATOTROPIC PITUITARY TUMOR.

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University of Minnesota, Minneapolis, MN
Diss Abstr Int (B); 42(1):30-B 1981

The MtTW15 rat pituitary tumor is a growth hormone (GH) and prolactin (PRL) secreting tumor which was induced in female Wistar/Furth rats by chronic estrogen administration. The purpose of this study was to determine the growth and functional responsivity of this tumor to estradiol-17 beta (E2) and medroxyprogesterone acetate (MPA) as well as their mechanism of action. Treatment of male and female tumor bearing rats began one wk after tumor inoculation and continued for seven wk. MPA promoted increases in GH production in male and female hosts but had little effect on the production of PRL. MPA promoted tumor growth in male tumor bearing rats as well. Estradiol-17 beta promoted tumor growth, inhibited GH production while having little effect on PRL. [3H]-E2 and [3H]-R5020 ligands were used to evaluate the estrogen and progestin cytosolic binding properties of MtTW15 tumors. Using a dextran charcoal separation method, it was found that all tumor cytosols bound [3H]-E2. In general, tumors from male rats bound more E2 than their female counterparts and steroid treatments affected E2 binding sites as well. Progestin binding was low in all groups; however, the highest values were obtained in tumors from E2 treated rats. But no evidence for progestin specific, high affinity binding sites was obtained. Further studies using a [3H]-MPA ligand demonstrated that the MtTW15 tumor contains cytosolic MPA binding sites. The concentration of sites was similar for tumors derived from male or female hosts, but was significantly reduced in tumors from MPA or E2 treated rats. In an additional study, four non-steroidal estrogen antagonists were administered for six wk to different groups of female tumor bearing rats. None of the antiestrogens significantly inhibited tumor growth. However, antiestrogens significantly inhibited tumor cytosolic E2 binding sites while increasing tumor GH production. Antiestrogens had little effect on the production of PRL. (no Refs)

27. 17 BETA-ESTRADIOL INHIBITS THE GROWTH OF THE TRANSPLANTABLE PITUITARY TUMOR MTF4 WHILE IT INCREASES THE GROWTH OF NORMAL PITUITARY (MEETING ABSTRACT).

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63rd Annual Meeting of the Endocrine Society, June 17-19, 1981, Cincinnati, Ohio. The Endocrine Society 385 pp., 1981.

The effect of 17 beta-estradiol (E2) on the growth of MtF4 transplantable pituitary tumor cells have been studied in vivo and in cell culture. Adult Fisher rats were injected SC with a suspension of cells prepared from a MtF4 tumor, then daily with oil +/-10 ug estradiol benzoate (E2B). During 7 experiments: in EB treated rats a tumor became palpable at the point of injection later than in control rat s; at the time of decapitation the tumor was smaller, or not detectable, and the pituitary wt was heavier than in control rats. The effects of E2B were similar in intact and castrated-adrenalectomized males or females; hypophysectomy did not modify the inhibition of tumor growth. Daily injections of 10 ug 17 alpha E2 or 50 ug dihydrotestosterone (DHT) had no significant effect on either normal pituitary or tumor growth. The effects of E2B were dose-dependent between 1 and 20 ug per day. A 50% competition was observed with 4 nM 17 beta-E2 and 40 nM estriol while no significant inhibition was produced by DHT, corticosterone and progesterone up to 10-6M. Culturing dispersed tumor cells and F4C1 clonal strain in the presence of 10(-8) to 10-5M 17 beta E2 for less than or equal to 7 days did not modify either the 3H-thymidine incorporation into DNA or the cell number. Pharmacological doses of E2 specifically inhibit the growth of MtF4 pituitary tumor cells in vivo but not in vitro. Normal pituitary does not seem to mediate this action. Although these tumor cells contained estrogen binding proteins, we can not specify at the moment whether the effect observed in vivo was direct or not. (no Refs)

28. PITUITARY CONTROL OF NITROSOMETHYLUREA-INDUCED RAT MAMMARY TUMOR GROWTH (MEETING ABSTRACT).

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63rd Annual Meeting of the Endocrine Society, June 17-19, 1981, Cincinnati, Ohio. The Endocrine Society 385 pp., 1981.

Twenty-four Sprague-Dawley female rats bearing 41 mammary carcinomas induced with N-nitrosomethylurea (NMU) were randomly divided among 3 treatment groups. Group I were ovariectomized and then 2 wk later implanted subcutaneously with silastic capsules containing 17 beta-estradiol 40 mg; after another 2 wk they were treated with hypophysectomy, estradiol implants remaining in place; after a further 2 wk they were killed and samples taken for serum prolactin and tumor progesterone receptor (PgR) assays. Group II were also ovariectomized and implanted with estradiol but were killed after 2 wk of estrogen treatment rather than being hypophysectomized. Group III were treated with ovariectomy alone and were killed 2 wk later. All tumors in Groups I and II increased 50% or more during estrogen treatment. After hypophysectomy, most (13/15) of the tumors in Group I remained static or regressed. For the tumors in Group I shown to be hormone-dependent by 50% or greater response to ovariectomy, the reduction in size after hypophysectomy was significant. However, the PgR levels after hypophysectomy remained as high as the PgR of tumors treated with estrogen alone. These observations suggest that estradiol has a direct effect on NMU-induced mammary carcinomas in stimulating PgR, but that estrogen stimulation of tumor growth is mediated by the pituitary gland . In a related experiment, 10 intact Sprague-Dawley female rats with 10 NMU-induced mammary tumors were treated for 3 wk with bromergocryptine 0.8 mg BID. Although serum prolactin in these rats fell significantly compared with untreated tumor-bearing controls, all tumors continued to grow. Thus, while NMU tumors are apparently dependent on the pituitary for growth, they are not solely dependent on prolactin. (no Refs)

29. OSSEOUS CHANGES AND OSTEOSARCOMAS IN MICE CONTINUOUSLY FED DIETS CONTAINING DIETHYLSTILBESTROL OR 17BETA-ESTRADIOL.

Highman B, Roth SI, Greenman DL
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JNCI; 67(3):653-662 1981

In a study on the long-term effects of dietary diethylstilbestrol or 17beta-estradiol on C3H mice, estrogens induced a proliferation of osseous trabeculae and increased the incidence and hastened the development of osteofibrotic areas in the sterna. There were 6 osteosarcomas, 2 having metastases, in 1,242 mice fed dietary estrogens over 360 days, but none in 356 untreated controls. These tumors were reviewed along with 4 early sternal osteosarcomas selected from 17 osteosarcomas only 1 in a control found thus far in two other ongoing case comparable studies. In at least one and possibly in two other early cases, tumors were associated with areas of osteofibrosis, and one tumor was probably associated with proliferation of bony trabeculae in the medullary cavity. (Author abstract) (21 Refs)

30. COMPARISON OF DEXAMETHASONE BINDING AND RESISTANCE TO INHIBITION OF INDUCED DIFFERENTIATION IN SUBCLONES OF MURINE ERYTHROLEUKEMIA CELLS.

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Biochem Biophys Res Commun; 101(3):823-829 1981

Dexamethasone (DEX) binding to subclones of the murine erythroleukemia cell line DS 19 having different sensitivities to DEX inhibition of hexamethylene bisacetamide (HMBA)-induced differentiation was characterized. Differentiation was assessed via benzidine staining for Hb. One clone (B6) was sensitive to DEX inhibition and the other clone (D1) was semi-resistant: D1 differentiation was only 40% inhibited at 1uM DEX. Scatchard analysis of the total amount of 3H-DEX specifically bound/intact cell yielded the following for B6 and D1, respectively: dissociation constant (Kd) = 5 +-1 and 14 +-1 nanoM, and number of binding sites/cell (n) = 34,000+-5,000 and 54,000+-5,000. Hence at less than 1 nanoM DEX the sensitive B6 cells bind more DEX due to greater affinity, but at greater than 10 nanoM the D1 cells bind more due to greater binding site number. Resistance of D1 cells to DEX inhibition appeared not to result from less DEX binding/cell. As in intact cells, the total amount of 3H-DEX specifically bound to B6 and D1 nuclei was nearly identical (12 femtomole/10(6) nuclei in the presence of 10 nanoM DEX). The time course of binding was also similar. For B6 cells cultured in 4 milliM HMBA and 10 nanoM 3H-DEX, where differentiation is inhibited, no additional retention of bound DEX occurred relative to that retained in the semi-resistant D1 cells. Resistance to DEX inhibition of HMBA-induced differentiation in the D1 subclone appeared not to be correlated with a reduction in the total or nuclear binding of DEX, but possibly with a defect at an event after binding of hormone-receptor complexes to chromatin. (16 Refs)

31. INFLUENCE OF PARITY STATUS OF FEMALE MICE ON GROWTH OF TRANSPLANTABLE CHEMICALLY INDUCED FIBROSARCOMA.

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Indian J Exp Biol; 19(5):405-407 1981

The effect of parity status on the growth of transplantable dimethylbenzdithionaphthene (DBDN)-induced fibrosarcomas was studied in female Swiss mice. The av duration of survival of multiparous females inoculated with 10(4) tumor cells was 40.6 days, compared with 30.0 days in virgin controls. Adoptive transfer of spleen cells from pregnant females caused mild inhibition of tumor growth. The sera from pregnant females inhibited the antitumor activity of spleen cells, while sera from primiparous females enhanced the antitumor activity of spleen cells. (17 Refs)

32. INHIBITION OF PROLIFERATION OF RAT PITUITARY TUMOR IN CULTURE BY THYROTROPIN RELEASING HORMONE (MEETING ABSTRACT).

Saito T, Yajima Y
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63rd Annual Meeting of the Endocrine Society, June 17-19, 1981, Cincinnati, Ohio. The Endocrine Society 385 pp., 1981.

The effects of hypothalamic hormones on cell proliferation were studied in rat pituitary tumor in culture, GH3. The cells 10(5) per dish, were incubated in Ham's F10 media in the presence of 10-6M of thyrotropin releasing hormone (TRH), luteinizing releasing hormone (LRH) or somatotropin-release inhibition factor, 0.05mg/ml or rat hypothalamic extract (HE) or vehicle for 24 hr. After incubation, incorporation of [3H]-thymidine (TI) into a cellular fraction precipitated by trichloracetic acid was determined by additional 1 hr incubation in the presence of [3H]-thymidine. The release of rat prolactin (rPRL) into the media during 24 hr incubation was assayed. The presence of TRH of 10-5M and of 10-6M during 24 hr incubation significantly suppressed TI to 76% and 38% of the control respectively. The cell count beginning from 1.2x10(5) per dish increased to 3.3x10(5) after 96 hr incubation in the control media. In the presence of TRH and HE, the cell counts increased to 2.9x10(5), respectively, significantly lower than that of the control. The elution pattern obtained by plotting the levels of activity coincided with that of [3H]-TRH applied to the column and with that of concentration of TRH determined by radioimmunoassay in each fraction. It is concluded that hypothalamic hormones, predominantly TRH, influence not only the release of rPRL from cultured rat pituitary tumor, GH3, but also the proliferation of its cells. (no Refs)

33. GLUCOCORTICOID EFFECTS ON THE GROWTH OF A HORMONE RESPONSIVE CHONDROSARCOMA (MEETING ABSTRACT).

McCumbee WD, McCarty KS, Lebovitz HE
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63rd Annual Meeting of the Endocrine Society, June 17-19, 1981, Cincinnati, Ohio. The Endocrine Society 385 pp., 1981.

The well-differentiated Swarm rat chondrosarcoma has been shown to be hormone responsive. Insulin and serum somatomedins stimulate transport processes and the synthesis of macromolecules in vitro. Tumors in hypophysectomized (HYPX) rats are atrophic. Tumor growth in HYPX rats is stimulated by the administration of growth hormone and cortisone. To further

assess the role of glucocorticoids in chondrosarcoma growth, tumors were grown in adrenalectomized, cortisone treated-adrenalectomized, amd sham operated rats and effects on tumor wt and ultrastructure were evaluated. In one experiment, operations were performed 20 days after rats were inoculated with tumor. Adrenalectomy markedly reduced the rate of tumor growth. In a second experiment rats were inoculated with tumor 10 days after surgery. Adrenalectomized rats were given daily im injections of cortisone or saline for 4 wk. Cortisone restored tumor growth in adrenalectomized rats. Ultrastructurally tumors from adrenalectomized rats showed a reduction in rough endoplasmic reticulum and decreased exocytotic/pinocytotic activity. These were restored by cortisone administration suggesting that glucocorticoids are influencing the promotion of synthetic activity and facilitate the release of secretory products. (2 Refs)

34. SENSITIVITY OF NORMAL HUMAN GRANULO-MONOCYTIC PRECURSORS AND CHRONIC MYELOID LEUKEMIA TO THE ACTION OF PROSTAGLANDIN AND LITHIUM (MEETING ABSTRACT).

Piacibello W, Aglietta M, Gavosto F
I Cattedra di Patologia Medica, Universita di Torino, Torino, Italy
Tumori; 67(2,SupplA):103 1981

We have investigated the sensitivity of normal granulo-monocytopoietic stem cells (CFU-GM) and CFU-GM from chronic myeloid leukemia patients (CML) to the effects of granulopoiesis modulating substances by means of in vitro culture in semisolid agar. In vitro growth of normal CFU-GM is stimulated by the addition to the culture system of indomethacin, an inhibitor of prostaglandin synthesis; on the other hand, addition of prostaglandin E1 (PGE1), in concentrations of 10(-10) M or more, significantly r educes the mumber of colonies produced in vitro. Lithium chloride, in concentrations from 1 to 5 mM, stimulates the growth of normal CFU-GM. The CFU-GM of CML, however, are totally insensitive to the addition of both lithium and indomethacin to the tissue culture and are only inhibited by very high concentrations (10(-7) M or greater) of PGE1. These findings, which indicate an altered response of CML cells to factors which regulate normal granulopoiesis, suggest mechanisms responsible for the pathogenesis of the uncontrolled expansion of the leukemic clone. (no Refs) (In ITA)

35. RELEASE OF PROSTAGLANDIN E (PGE) FROM SPLEEN MACROPHAGES AND RELATIONSHIP TO SUPPRESSION OF T LYMPHOCYTE STIMULATION DURING TUMOR GROWTH IN MICE (MEETING ABSTRACT).

Deimann W, Seitz M, Gemsa D
No affiliation given
Immunobiol; 159(1/2):102 1981

Lymphocyte proliferation has been found to be reduced during tumor growth. Several suppressive mechanisms have been implicated, including an active cellular suppression by stimulated macrophages. To characterize the function of macrophages in lymphocyte stimulation during tumor growth, DBA/2 mice were ip injected with 1 x 10(6) syngeneic Eb or ESb lymphoma cells which were grown free of lactate dehydrogenase virus and mycoplasma. Seven days after tumor implantation, the wt of spleens was doubled and the macrophage content increased from 9 to 23%. Spleen cells from tumor-bearing mice spontaneously released 5 to 10 x more PGE than cells from control mice. Since an enhanced release of PGE has previously been shown to be accompanied by a suppression of lymphocyte proliferation, the concanavalin A (Con A) and lipopolysaccharide (LPS) response of spleen cells was examined. It was found that only the proliferative response of lymphocytes to Con A, but not to LPS, was strongly reduced in tumor-bearing animals. The Con A stimulation could be restored by two procedures, either by addition of indomethacin, an inhibitor of prostaglandin synthesis, or by a passage of spleen cells over rayon wool columns. Further support for PGE as a suppressive compound was obtained when synthetic PGE in amounts found in spleen cell cultures of tumor-bearing mice or admixture of PGE-releasing peritoneal macrophages elicited by inflammatory stimuli similarly suppressed the lymphocyte response of Con A. In contrast to spleen cells, no enhanced release of PGE was detected in cultures of mesenterial lymph node lymphocytes from tumor-bearing animals and the Con A response was not suppressed but significantly elevated in most instances when compared to control cells. These findings demonstrate that during growth of transplantable tumors in mice, there occurred in the spleen an accumulation of macrophages, of which a strongly adherent population participated in the suppression of T lymphocyte proliferation via release of PGE. (no Refs)

EXOGENOUS AGENTS AFFECTING DIFFERENTIATION

36. CHANGES IN CELL SURFACE PROTEINS DURING DIFFERENTIATION OF MOUSE EMBRYONAL CARCINOMA CELLS.

Howe CC, Solter D
Wistar Inst. Anatomy and Biology, 36th St. at Spruce, Philadelphia, PA, 19104
Dev Biol; 84(1):239-243 1981

The cell surface proteins of teratocarcinoma-derived embryonal carcinoma cells (ECC), of parietal endoderm (Pys-2 and F9-AC cl 9), and of fibroblasts (OTT6050f) were radioiodinated by a lactoperoxidase method and analyzed by two-dimensional gel electrophoresis. The combined electrophoretic profiles from a number of ECC lines allowed the determination of eight ECC-unique polypeptides. Parietal endoderm and fibroblast expressed their own unique polypeptides. The two parietal endoderm-specific polypeptides are identical to the subunits of laminin. Retinoic acid-induced differentiation of one ECC line (F9) resulted in the disappearance of polypeptides specific for ECC and the appearance of those specific for the parietal endoderm. (Author abstract) (34 Refs)

37. STUDIES ON THE MECHANISM OF INDUCTION OF EMBRYONAL CARCINOMA CELL DIFFFRENTIATION BY RETINOIC ACID.

Sherman MI, Matthaei KI, Schindler J
Roche Inst. Molecular Biology, Nutley, NJ, 07110
Ann NY Acad Sci; 359:192-199 1981

The effect of retinoic acid (RA) on differentiation of embryonal carcinoma (EC) cells was studied. RA-resistant PCC4-azal EC cells were cultured with RA at 10(-5) M. After 2-wk culturing, mice were inoculated sc with control or RA-treated EC cells. All mice inoculated with control EC cells developed tumors within 10-12 days, while no tumor growth was detected in 6/8 mice inoculated with RA-treated cells. Histological examination of tumors in two other mice showed teratocarcin-

oma. Mutagen treatment and clonal selection of EC cells that fail to respond to RA indicated that these cells have lost the capacity to differentiate when subjected to other manipulations which stimulate differentiation of the parental EC cells. (23 Refs)

38. RETINOID STIMULATES EPIDERMAL OUTGROWTH OF PIG SKIN EXPLANTS.

Aoyagi T, Kamigaki K, Kato N, Fukaya T, Iizuka H, Miura Y
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J Dermatol (Tokyo); 8(3):197-205 1981

The effects of retinoids on epidermal outgrowth and mitotic index of the outgrowing epidermis was investigated using an explant culture system of pig epidermis. Ro 10-1670 significantly stimulated epidermal outgrowth, while Ro 10-9359 did not. No significant changes in the mitotic index were noted after exposure to either retinoid. Cytochalasin B and colchicine markedly inhibited the stimulatory effect of Ro 10-1670, while hydroxyurea or cycloheximide did not completely block this stimulatory effect. The stimulatory effect of Ro 10-1670 on epidermal outgrowth was completely blocked by cytochalasin B during the migratory period. In the presence of Ro 10-1670, keratinization of the outgrowing epidermis was inhibited and no overlapping of epidermal cells was observed. These results suggest that Ro 10-1670 stimulated outgrowth, particularly migratory outgrowth, of epidermal cells, resulting in reduced keratin formation. (38 Refs)

39. CHARACTERIZATION OF RETINOIC ACID-INDUCED ALTERATIONS IN THE PROLIFERATION AND DIFFERENTIATION OF A MURINE AND A HUMAN MELANOMA CELL LINE IN CULTURE.

Lotan R, Neumann G, Lotan D
Dept. Biophysics, Weizmann Inst. Science, Rehovot, Israel
Ann NY Acad Sci; 359:150-170 1981

To evaluate the biochemical changes induced by retinoids, S91 mouse melanoma cells and human Hs939 melanoma cells were incubated with retinoic acid (RA). RA caused a dose-dependent inhibition of the growth of S91 cells in liquid medium and reduction of colony-formation in semi-solid medium. Only continuous presence of RA caused a suppression of their anchorage-independent growth. Exposure of S91 cells to RA decreased the proportion of cells in the S and G2 phases of the cell cycle with a concomitant increase in G1 cells; since the fraction of cells in G1 phase did not exceed 80%, RA did not block cells at this phase but rather reduced the rate of their entry into S phase. There was a significant reduction in the DNA content and a 25% decrease in the RNA content. Treatment with RA did not change the protein content. RA caused progressive reduction of the incorporation of labeled DNA, RNA and protein precursors. RA induced an increase in the melanin production in both S91 and Hs939 cells, but stimulation of melanogenesis was not preceded by an elevation in the level of cyclic AMP. Testing of 27 different retinoids showed that the compounds with a free carboxyl group at carbon 15 possessed the most pronounced ability to inhibit cell growth and to bind to RA-binding protein. (66 Refs)

40. EARLY CHANGES IN PHOSPHATIDYLCHOLINE METABOLISM IN HUMAN ACUTE PROMYELOCYTIC LEUKEMIA CELLS STIMULATED TO DIFFERENTIATE BY PHORBOL ESTER.

Cassileth PA, Suholet D, Cooper RA
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Blood; 58(2):237-243 1981

The HL-60 leukemia cell line derived from a human acute promyelocytic leukemia is stimulated to differentiate into macrophages within 24-48 hr after exposure to the phorbol ester, 12-O-tetradecanoylphorbol-13-acetate (TPA). We studied early alterations (within 90 min of exposure to TPA) in phosphatidylcholine metabolism in HL-60 cells and found that phosphatidylcholine synthesis by methylation of phosphatidylethanolamine was inhibited in a dose-dependent fashion. In contrast, synthesis of phosphatidylcholine from endogenous choline was enhanced and correlated inversely with the degree of inhibition of the methy lation pathway. Phorbol ester congeners of TPA caused similar alterations in phosphatidylcholine metabolism in direct relationship to their capacity to induce differentiation in HL-60 cells. Perturbation of phosphatidylcholine metabolism is an early membrane event in TPA-induced HL-60 cell differentiation. (Author abstract) (44 Refs)

41. PHORBOL-ESTER INDUCED PROLIFERATION IN HUMAN T-CELL LEUKEMIA (MEETING ABSTRACT).

Vyth-Dreese FA, De Vries JE, Spits H, Van Der Reijden JH
No affiliation given
Immunobiol; 159(1/2):29 1981

The mitogenic reactivity of peripheral blood lymphocytes (PBL) from 5 patients with T-cell chronic lymphocytic leukemia (T-CLL) and 6 patients with acute T-cell leukemia (T-ALL) were investigated. The PBL of only 2T-CLL patients reacted to phytohemagglutinin (PHA); 3H-thymidine (3H-TdR) incorporation in the PBL of one of these patients followed normal dose-response curves, whereas the PBL of the other patient were only found to proliferate after prolonged incubation at suboptimal PHA concentrations. The non-responsiveness of the PBL of the other 9 patients could not be attributed to different dose-response characteristics or altered kinetics. Stimulation of these PBL with PHA in the presence of the tumor promotor 12-O-tetradecanoyl phorbol 13-acetate (TPA) resulted in the induction of proliferation in 3 out of 3 T-CLL and 4 out of 6 T-ALL patients. The extent of 3H-TdR incorporation varied from 2500 to 52,000 cpm per culture in the individual patients and was found to depend on the mitogen dose used. Furthermore, addition of TPA induced an increase in the PHA response of the PBL of the 2 T-CLL patients which showed to respond to PHA. A direct correlation was observed between the induction and enhancement of proliferation and the generation of Interleukin 2 (IL2) in the supernatant of PHA + TPA stimulated cultures of 6 out of 7 leukemic cell populations tested. IL2, which acts as a second signal in normal-T-cell proliferation, was produced only in minor amounts after stimulation with TPA alone. The results indicate that 1) the lack of PHA reactivity of leukemic T cell cultures can be attributed to the failure of IL2 production, 2) stimulation with TPA results in borderline 3H-TdR incorporation as well as IL2 production, 3) TPA strongly induces IL2 production in the presence of PHA, which may act as an activating signal. (no Refs)

42. INFLUENCE OF LITHIUM ON PROLIFERATION OF HEMATOPOIETIC STEM CELLS.

Gallicchio VS, Chen MG
Dept. Therapeutic Radiology, Yale Univ. Sch. Medicine, 333 Cedar St., New Haven, CT, 06510
Exp Hematol; 9(7):804-810 1981

We studied the effect of lithium on normal marrow hematopoiesis by determining the numbers of pluripotential (CFUS) and committed stem cells (CFUC, CFUE and BFUE) after in vitro and in vivo exposure to lithium. In the presence of 1 meq/L lithium in vitro, marrow CFUS and CFUC were increased; higher concentrations (greater than or equal to 5 meq/L) were inhibitory. Marrow CFUE and BFUE were decreased at concentrations of lithium greater than or equal to 0.5 meq/L. In vivo (0.5-5.0 meq/L ip), lithium produced similar results to those obtained in vitro with striking CFUC enhancement. Serum from these lithium-treated mice contained increased colony stimulating factor (CSF). In the Dexter continuous marrow culture system, lithium stimulated increased CFUC production from the non-adherent fraction. These in vitro and in vivo studies document lithium's ability to modulate hematopoiesis by influencing pluripotential and committed stem cell proliferation and differentiation towards granulopoiesis, apparently at the expense of erythropoiesis. Mechanisms of this modulation are discussed. (Author abstract) (25 Refs)

OTHER RELATED STUDIES

43. INHIBITION OF EPIDERMAL GROWTH FACTOR BINDING TO MOUSE EMBRYONAL CARCINOMA CELLS BY PHORBOL ESTERS MEDIATED BY SPECIFIC PHORBOL ESTER RECEPTORS.

Salomon DS
Lab. Pathophysiology, NCI, NIH, Bethesda, MD, 20205
J Biol Chem; 256(15):7958-7966 1981

Exposure of OTT-6050 mouse embryonal carcinoma (EC) cells to 12-O-tetradecanoyl phorbol-13-acetate (TPA) at 37 C results in an 80 to 90% reduction within 10 to 15 min in the specific binding capacity for [125I]-epidermal growth factor (EGF). At 37 C max specific binding of [125I]EGF occurs at 1 hr and is followed by a reduction in the binding of [125I]EGF. This decrease is temperature dependent and is not affected by TPA treatment. Scatchard analysis of the binding data indicates that the TPA-induced reduction in [125I]EGF binding is due to a decrease in the number of available EGF receptors/cell, without any change in the affinity of the receptors for EGF. Other tumor-promoting phorbol esters also inhibit [125I]EGF binding. The inhibitory effect of tumor-promoting phorbol esters on EGF binding is mediated by the interaction of these compounds with a specific receptor which is distinct from the EGF receptor. Binding of [20-3H]phorbol 12,13-dibutyrate (PDB) has been measured on EC cells, and it is specific and saturable. Saturation of specific [3H]PDB binding occurs between 25 and 35 nM at either 37 or 4 C, which correlates with the concentration of PDB producing half-max inhibition of [125I]EGF binding (50 nM). Other tumor-promoting phorbol esters inhibit [3H]PDB binding in a dose-dependent manner which parallels their activity in inhibiting [125I]EGF binding to its receptor and their tumor promoting activity in vivo. TPA is the most potent phorbol ester in competing for [3H]PDB binding with half-max inhibition at 2.5 nM. Specific binding of [3H]PDB is not directly affected by compounds such as EGF, dexamethasone, retinoic acid, insulin, transferrin, and vasopressin, but EGF can indirectly affect it. The ability of tumor-promoting phorbol esters to inhibit EGF binding may be mediated by a specific phorbol ester receptor. Furthermore, EGF may modulate the processing of phorbol ester receptors by preventing the presumptive internalization of the phorbol ester-receptor complex. (Author abstract) (46 Refs)

44. MEASUREMENT OF INSULIN-LIKE GROWTH FACTOR II BY A SPECIFIC RADIORECEPTOR ASSAY IN SERUM OF NORMAL INDIVIDUALS, PATIENTS WITH ABNORMAL GROWTH HORMONE SECRETION, AND PATIENTS WITH TUMOR-ASSOCIATED HYPOGLYCEMIA.

Daughaday WH, Trivedi B, Kapadia M
Metabolism Div., Barnes and Wohl Hosp., 660 South Euclid Ave., St. Louis, MO, 63110
J Clin Endocrinol Metab; 53(2):289-294 1981

A radioreceptor assay for insulin-like growth factor II (IGF-II) is described in which [125I]IGF-II is bound by rat placental membranes. IGF-I and other insulin-related peptides have less than 1% of the potency of IGF-II in displacing [125I]IGF-II. IGF-II is extracted from its serum binding protein with a simple acid-ethanol step. After neutralization with Tris base, the extract is introduced directly into the radioreceptor assay. Results are expressed in terms of a reference serum with an assigned potency of 1 U/ml. By this method, serum from normal adults, 20-69 yr of age, had a mean (+- SE) IGF-II activity of 0.73 +-0.03; higher concentrations were noted in adults more than 70 yr of age (1.05 +-0.05), in cord serum (1.55 +-0.24), and in short children with normal GH secretion (0.88 +-0.42). In hypopituitary dwarfism, serum IGF-II activity was reduced (0.50 +-0.05), but in acromegaly, it was not increased (0.77 +-0.06). In 10 of 14 serum samples from patients with tumor-related hypoglycemia, the IGF-II exceeded the normal 97.5% confidence limits. In 8 of these sera, IGF-I by RIA was low, and in 5, it was essentially unmeasurable. These results with a new radioreceptor assay for IGF-II provide additional evidence that the regulation of this serum peptide differs from that of IGF-I. (Author abstract) (16 Refs)

45. ANTIPROLIFERATIVE EFFECTS OF RETINOIDS RELATED TO THE CELL CYCLE-SPECIFIC INHIBITION OF ORNITHINE DECARBOXYLASE.

Russell DH, Haddox MK
Dept. Pharmacology, Univ. Arizona Coll. Medicine, Tucson, AZ, 85724
Ann NY Acad Sci; 359:281-297 1981

A review is presented of the major experimental evidence obtained in Chinese hamster ovary (CHO) cells which supports the thesis that retinoid analogs affect the transcription of messenger RNA for ornithine decarboxylase (ODC) and thus arrest cells in G1 prior to the cell cycle-specific expression of ODC. Some of the areas explored include concentration-dependent inhibition of asynchronous CHO cell growth by retinoids; role of flow microfluorimetry in this inhibition; dose-dependent inhibition of O DC induction during G1 progression; lack of effect of retinol on activation of cyclic AMP-dependent protein kinase during G1; effect of RNA and protein synthesis in synchronized CHO cells; ODC expression after retinol block by actinomycin D; effect of retinol on other parameters of polyamine biosynthesis and accumulation and implications of retinol studies of synchronized CHO cells. Retinoids only inhibit the induction of ODC activity during the first 2-3 hr of G1 progression. It is postulated that ODC is a requirement for G1

progression and the antiproliferative properties of retinoids are related to the specific ability to inhibit this expression. Since retinoids do not markedly alter the rate of protein synthesis, their ability to inhibit ODC may be related to their ability to inhibit messenger RNA synthesis for ODC. (150 Refs)

46. SODIUM BUTYRATE STIMULATES HCG AND HCG-ALPHA PRODUCTION IN A HUMAN CHORIOCARCINOMA CELL LINE (BEWO).

Kinoshita F, Nakai Y, Nikaido O, Imura H
Second Div., Dept. Medicine, Kyoto Univ. Sch. Medicine, Kyoto 606, Japan
J Cancer Res Clin Oncol; 100(3):321-324 1981

The effect of sodium butyrate on cell growth and hormone production in BeWo, a trophoblastic cell line and in HeLa S3, a nontrophoblastic cell line, was investigated. In HeLa S3, 2 milliM SB inhibited cell growth but stimulated production of human chorionic gonadotropin-alpha (hCG-a), found both in the medium and cell extract. In BeWo, 2 milliM SB stimulated hCG and hCG-a production, even though cell growth was decreased. Intracellular hCG increased fivefold after 48 hr of exposure to SB, and increased further after 96 hr exposure to SB. These results suggest that the regulation of hormone production in trophoblastic and nontrophoblastic tumor cells probably does not differ. (7 Refs)

47. ROLE OF AEROBIC CONDITIONS IN THE CONTROL OF CELL PROLIFERATION.

Karlberg I, Lindahl-Kiessling K, Low H, Mattsson A
(c/o Lindahl-Kiessling), Inst. Zoophysiology, PO Box 560, S-751 22 Uppsala, Sweden
Int Arch Allergy Appl Immunol; 65(3):250-256 1981

Our experiments demonstrate that proliferation of human lymphocytes as well as fibroblasts is inhibited by oxygen. It is not a toxic effect and it has not been possible to find an intermediary link in intracellular glycolysis, oxidative metabolism, or macromolecule synthesis. Accessory cells may reduce the inhibitory action of oxygen, thereby exerting an influence on the growth rate. In view of recent hypotheses on the mediation of hormone effects via a membrane dehydrogenase system our results may indicate that also the mitotic activation is dependent on the redox state of the membrane for the function of a proton gradient across the plasma membrane itself, delivering energy for glucose and amino acid transport. (Author abstract) (21 Refs)

48. A STUDY OF THE RELATIONSHIP BETWEEN THE MITOTIC ACTIVITY OF CANCER CELLS AND VARIOUS FACTORS OF GASTRIC CANCER PATIENTS.

Tabuchi Y, Takiguchi Y, Murayama Y, Saitoh Y
First Dept. Surgery, Kobe Univ. Sch. Medicine, Ikuta-ku, Kobe 650, Japan
Nippon Geka Gakkai Zasshi; 82(1):42-50 1981

An in vivo stathmokinetic method was used to analyze the mitotic activity of cancer cells from 47 gastric cancer patients. The effect of cellular reaction in cancer tissues on the mitotic activity of cancer cells was examined. The relationship between general factors of the patients such as immunologic activity, changes of protein metabolism and the mitotic activity of cancer cells was also examined. The following results were obtained. An inverse correlation was found between the mitotic activity of cancer cells and the degree of lymphoid cell infiltration in cancer tissues. There was a tendency to a decrease in the mitotic activity of cancer cells paralleling an increase in the degree of delayed skin hypersensitivity to purified protein derivative and phytohemagglutinin and in the number of lymphocytes in peripheral blood. Inverse correlations between the mitotic activity of cancer cells and the values of Hb, hematocrit, albumin and ratio of albumin/globulin were found. Cancer cells showed a tendency to an increase in mitotic activity paralleling an increase in alpha1 and alpha2 globulin of peripheral blood. Highly significant correlations between the mitotic activity of cancer cells and the activity of lactic dehydrogenase (LDH) and its isoenzymes (LDH 3-5) in peripheral blood were observed. These results suggest the possibility that the mitotic activity of cancer cells is regulated by the infiltrating lymphoid cells in cancer tissue and promoted by impairment of cellular immunity. Moreover, it may be estimated that protein metabolism and the function of RBC formation are impaired and that the activity of LDH derived from cancer cells increases in peripheral blood, in parallel with the increase in the mitotic activity of cancer cells. (50 Refs) (In JPN)

49. TOWARD A NUTRITIONAL CONCEPT OF HOST RESISTANCE TO MALIGNANCY AND INTRACELLULAR INFECTION.

Murray J, Murray A
Dept. Medicine, Univ. Minnesota, Box 385, Mayo Building, University Hospitals, Minneapolis, MN, 55455
Perspect Biol Med; 24(2):290-301 1981

The effects of starvation on resistance to malignancy and intracellular infection are discussed. The authors cite various studies showing that starvation under some conditions increases resistance in man and animals. Interferon and chalones are cited as substances that control growth. Mechanisms are suggested in which control substances induced by starvation bring about lowered circulation and conversion of thyroxin, causing a lowered metabolism rate. Chalones, acting with adrenalin, raise levels of cycl ic AMP. This favors degradative energy-producing processes at the expense of mitosis. A reduction in mechanisms producing RNA and DNA makes a cell less favorable as a host for viral infection. Some evidence suggests that factors in the serum of starving animals may suppress malignancy in normally-fed animals. The need to define the nutritional requirements for optimum resistance to cancer and infection is expressed. Caution is urged in modifying the diets of tribal societies with low nutritional standards. Vigorous exploration of dietary manipulation and the use of natural growth inhibitors for the management and prevention of malignancies is proposed. (67 Refs)

50. PHYSIOLOGICAL ADAPTATION AT THE MOLECULAR LEVEL: THE FRONTIER WHERE RESEARCH ON DIFFERENTIATION AND MALIGNANCY MEET.

Potter VR
McArdle Memorial Lab., Univ. Wisconsion Medical Sch., Madison, WI, 53706
Perspect Biol Med; 24(4):525-542 1981

Research on physiological adaptation that combines the holistic and reductionist approaches was believed to be the key to understanding differentiation and malignancy. The concept that the most significant aspect of the genetic program for differentiation is the development of a whole organism capable of physiological adaptation was proposed. In other words, the capability is a condition in which every cell lineage contains a few or many cells that are capable of fairly rapid shifts in th amount and activity of the enzymes, transport mechanisms, re-

ceptors, and proliferative essentials, all designed to make the total organism physiologically adapted to a changing environment. Normal differentiation was considered to be a process that is directed toward competence for physiological adaptation, while cancer cells fail to carry out the program. It was suggested that a change in the net rate of synthesis of one or more types of messenger RNA together with a change in the corresponding enzyme amount and activity be considered to represent physiological adaptation. Similarly, the ability to regulate cell division, was thought to be an important aspect of physiological adaptation as related to differentiation and malignancy; however regulation of cell division is the sixth line of defense in the hierarchy of six cellular defense lines available during physiological adaptation at the molecular level. (43 Refs)

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CELL AND SERUM DERIVED GROWTH EFFECTORS

1. INCREASED PHOSPHOTYROSINE CONTENT AND INHIBITION OF PROLIFERATION IN EGF-TREATED A431 CELLS.

Gill GN, Lazar CS
Dept. Medicine, Div. Endocrinology, Univ. California San Diego, Sch. Medicine, La Jolla, CA, 92093
Nature; 293(5830):305-307 1981

The effect of epidermal growth factor (EGF) on phosphotyrosine content of exponentially growing A431 human epidermoid carcinoma cells was determined by labelling cells to equilibrium with 32P-orthophosphate, treating replicate plates 3 hr with EGF, and measuring phosphotyrosine. EGF increased

HE 20.3173/2:

phosphotyrosine content 7x from 0.02 to 0.14% of cellular phosphoamino acids, indicating that EGF activates tyrosine-specific protein kinase activity in vivo as it does in vitro and the kinase activity is an important component of the mechanism of EGF action. In contrast to Rous sarcoma virus (RSV) transformation, EGF inhibits replication of A431 cells. No growth was apparent when A431 cells were maintained with saturating concentrations of EGF over a 14-day period. Affinity of EGF receptors for EGF was inversely related to cell density. The measured equilibrium dissociation constant, Kd, for EGF binding at low and high cell densities was higher than the half-maximally effective concentration, EC50, of EGF required to inhibit cell proliferation at that cell density (7x at 500 cells/square cm and 4x at 15,000 cells/square cm). Occupancy of only a fraction of EGF receptors seems to be required for growth inhibition. Dose-response of growth inhibition was strongly affected by tissue culture substratum. (36 Refs)

2. TISSUE CULTURE OF HUMAN EPITHELIAL CELLS FROM BENIGN COLONIC TUMORS.

Friedman EA, Higgins PJ, Lipkin M, Shinya H, Gelb AM
Lab. Gastrointestinal Cancer Res., Memorial Sloan-Kettering Cancer Center, New York, NY, 10021
In Vitro; 17(7):632-644 1981

Human colonic epithelial cells from three classes of benign tumors have been reproducibly cultured free of fibroblasts for 8 wk using a supplemented Medium 199 (M 199S). The cultured colonic cells were identified as epithelial by the presence of junctional complexes (tight junctions, gap junctions, and desmosomes), a brush border on the apical surface, keratin fibrils, and by both a close-packed columnar or cuboidal morphology and the capability to transport water and ions to form hemicysts. Colony formation was initiated by groups of epithelial cells, not by single cells, and was inhibited by cocultivation with either lethally irradiated 3T3 cells or human diploid fibroblasts. Enhancement of epithelial colony formation was observed following culture on nonadherent, 'floating' substrates compared with substrates attached directly to the bottom of the culture dish. Replication of epithelial cells in M 199S from the class of benign colonic tumors least prone to malignancy, the tubular, was significantly enhanced by *epidermal growth factor* (EGF). In contrast, EGF did not stimulate the growth of cells in M199S from the other classes of benign tumors, the villotubular and the villous, which exhibit more malignant potential. These data imply that premalignant colonic epithelial cells lose responsiveness to growth modulation by EGF as they progress toward frank carcinoma. (Author abstract) (40 Refs)

3. EPIDERMAL CELL (KERATINOCYTE)-DERIVED THYMOCYTE-ACTIVATING FACTOR (ETAF).

Luger TA, Stadler BM, Katz SI, Oppenheim JJ
NIH, Natl. Inst. Dental Res., Bldg. 30, Room 324, Bethesda, MD, 20205
J Immunol; 127(4):1493-1498 1981

In order to determine whether keratinocytes play a role in the modulation of the immune response, we investigated the murine keratinocyte cell line Pam 212. In culture these cells generate a substance with a biologic activity that greatly enhances phytohemagglutinin-induced thymocyte proliferation. We have, therefore, called this substance epidermal cell thymocyte-activating factor (ETAF). This keratinocyte-derived supernatant activity is mainly produced at the onset of the logarithmic growth phase and is directly mitogenic for murine thymocytes. Although ETAF by itself exhibits no T cell growth factor activity, ETAF enhances interleukin 2 production by mitogen-stimulated murine spleen cells. Murine ETAF is not genetically restricted and lacks species specificity since it decreases lectin-induced proliferation of human peripheral blood lymphocytes (as well as murine spleen cells) and also enhances the production of human interleukin 2. The factor has a mol wt between 15,000 and 25,000 as determined by gel filtration and elutes as a single peak from anion exchange chromatography columns. The activity is maintained mainly at alkaline pH and is rapidly destroyed at temperatures above 60 C. These observations suggest that epidermal cells may interact with the immune system by elaborating nonspecific factors that modulate lymphocyte proliferation and augment lymphokine production. (Author abstract) (41 Refs)

4. EFFECTS OF 12-O-TETRADECANOYL PHORBOL-13-ACETATE AND EPIDERMAL GROWTH FACTOR ON THE PROLIFERATION OF HUMAN MUTANT FIBROBLASTS IN VITRO.

Gansler T, Kopelovich L
(c/o Kopelovich), Memorial Sloan-Kettering Cancer Center, 1275 York Ave., New York, NY, 10021
Cancer Lett; 13(4):315-323 1981

The role of cell density in the response of cultured skin fibroblasts (SF) from individuals with hereditary adenomatosis of the colon and rectum (ACR) and from normal individuals to 12-O-tetradecanoyl phorbol-13-acetate (TPA) and to epidermal growth factor (EGF) was examined. The effect of TPA and EGF on the morphology of cell clones was also studied. Treatment of high density (6 x 10(4) cells/square cm) ACR-SF cultures with TPA produced dose-dependent stimulation of cell proliferation over the entire (0.5-100 nanog/ml) range tested. Addition of TPA to sparse (4.75 x 10(3) cells/square cm) cultures resulted in biphasic dose-response, with max inhibition at 2.0-10 nanog/ml. Similar results were found with normal human SF, primarily in sparse cultures, but these cells were more sensitive than ACR-SF. Normal SF in dense culture were not responsive to TPA stimulation. EGF (0.01-10 nanog/ml) was mitogenic in both sparse and confluent ACR-SF cultures in a dose-dependent manner. At EGF levels above 50 nanog/ml, however, inhibition of cell proliferation became apparent. Normal SF responded similarly. TPA exposure induced formation of dendritic-like cells with irregular thin cytoplasmic processes in both cell type s. ACR-SF exposed to EGF were more densely packed and spindle-shaped, with more parallel orientation in the periphery. The results suggested that EGF and TPA share one of two existing TPA receptors; the higher affinity (unshared) receptor becomes saturated at ligand concentrations below 10 nanog/ml. TPA inhibition in sparse culture reflects the extent of neoplastic transformation; the less sensitive tumor cells apparently lack the higher affinity receptors. (26 Refs)

5. TELEOCIDIN, A POSSIBLE NATURALLY OCCURRING TUMOR PROMOTER FROM STREPTOMYCES, MODULATES ACTIONS OF EPIDERMAL GROWTH FACTOR ON RAT HEPATOMA CELLS.

Kaneko Y, Imai Y
First Dept. Medicine, Univ. Tokyo, 7-3-1, Hongo, Bunkyo-ku, Tokyo
Life Sci; 29(15):1571-1576 1981

Epidermal growth factor (EGF) stimulated the proliferation of rat AH66 hepatoma cells in a low-serum culture. Teleocidin, a toxic substance isolated from Streptomyces, inhibited the binding of [125I]EGF to cellular receptors and antagonized the

mitogenic action of EGF. Insulin binding to AH66 cells was not affected by teleocidin, suggesting that the effect of teleocidin might be selective to the action of EGF. However, the inhibition of EGF binding by teleocidin was a transient phenomenon, and AH66 cells escaped from and became refractory to the teleocidin-inhibition of EGF binding after a prolonged treatment with teleocidin. In addition, teleocidin seemed to have no remarkable effect on the internalization of EGF-receptor complex. These results appeared to indicate that the effect of teleocidin on the mitogenic action of EGF might be related with a pathway of EGF action following the interaction of EGF with its receptors. (Author abstract) (26 Refs)

6. SIMILARITIES BETWEEN FIBROBLAST-DERIVED GROWTH FACTOR AND PLATELET-DERIVED GROWTH FACTOR.

Dicker P, Pohjanpelto P, Pettican P, Rozengurt E
Imperial Cancer Res. Fund, Lincoln's Inn Fields, London WC2A 3PX, England
Exp Cell Res; 135(1):221-227 1981

Platelet-derived growth factor (PDGF), one of the most potent mitogens in serum for non-transformed cells, shares many biological and physical properties with fibroblast-derived growth factor (FDGF), a polypeptide produced by BHK cells transformed by SV40. Thus FDGF and PDGF have biological activity which is recoverable from sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis, at positions indicating similar mol wt. Further, the biological activity of both factors is heat-stable but sensitive to mercaptoethanol. FDGF and PDGF have similar abilities to induce DNA synthesis synergistically in the presence of either insulin, epidermal growth factor (EGF), vasopressin or colchicine. In contrast to other growth factors, (i) either FDGF or PDGF can induce DNA synthesis in the absence of other mitogens in 3T3 cells maintained in serum-free medium and (ii) a transient exposure of cultures to FDGF or PDGF causes a persistent stimulation of DNA synthesis. Either FDGF or PDGF enhances colony formation of non-transformed cells cultured in suspension in the presence of EGHF and serum. FDGF is not PDGF adsorbed by SV40-BHK cells from serum, since SV40-BHK cells plated and grown in the absence of serum still produce FDGF. In view of the similarities between PDGF and FDGF, we suggest that they may belong to the same family of growth factors. (Author abstract) (20 Refs)

7. INCREASED LEVELS OF NEURON-SPECIFIC ENOLASE IN PC12 PHEOCHROMOCYTOMA CELLS AS A RESULT OF NERVE GROWTH FACTOR TREATMENT.

Vinores SA, Marangos PJ, Parma AM, Guroff G
Section on Intermediary Metabolism, Lab. Developmental Neurobiology, Natl. Inst. Child Health and Human Development, Bethesda, MD, 20205
J Neurochem; 37(3):597-600 1981

Treatment of PC12 pheochromocytoma cells with nerve growth factor (NGF) resulted in increased levels of neuron-specific enolase (NSE). Neither insulin, growth hormone, cytochrome c, nor sodium butyrate increased NSE levels. Epidermal growth factor (EGF) did increase NSE levels, although not to the same extent as NGF. As little as 1 ng/ml NGF induced the max increase in NSE. As PC12 cells increased in density, the NSE levels increased even in untreated cells. (Author abstract) (24 Refs)

8. AGE-DEPENDENT INHIBITION OF GROWTH OF MOUSE NEUROBLASTOMA CELLS BY MOUSE BRAIN EXTRACT.

Endoh Y, Takeda S, Matsuzawa T
Dept. Radiology and Nuclear Medicine, Res. Inst. Tuberculosis and Cancer, Tohoku Univ., 4-1 Seiryo-machi, Sendai 980, Japan
Exp Cell Biol; 49(5):272-277 1981

In the developing mouse brain, inhibition of cell proliferation and cell death occur naturally. In order to study the factors which control growth and differentiation of neural cells, we added brain extract of mice of various ages to N-18 mouse neuroblastoma cell cultures. Brain extract from newborn mice inhibited N-18 cell growth and, at high concentrations, induced cell death. The colony-forming ability of N-18 cells was also inhibited by brain extract. This inhibitory effect of brain extract on the colony-forming ability increased with fetal age and became most significant at birth, then gradually decreased. Brain extract of 19-day embryos promoted the neurite outgrowth of N-18 cells at low concentrations, at which cell growth was not affected. On the other hand, brain extract of adult mice had no effect on the growth and morphology of cells at the same concentration. (Author abstract) (18 Refs)

9. ANGIOGENESIS AND NEOPLASIA.

Gullino PM
NCI, Bethesda, MD, 20205
N Engl J Med; 305(15):884-885 1981

A possibility that prevention of capillary proliferation could prevent tumor growth is discussed. Clinical application of anti-angiogenesis in the arrest of tumor growth is not feasible due to insufficient knowledge of the angiogenic process. Since the interstitial fluid of solid tumors in vivo is highly angiogenic, the tumor cells must release angiogenesis factor (AF) into the environment. Attempts at purification of AF have been unsuccessful. If AF exists, it is probably a trigger in a cascade of events that may be initiated under a variety of conditions. When fluids bathing a tumor acquire angiogenic activity, the presence of a tumor is revealed; neoplastic transformation may be suspected if AF is found in a tissue in which it is normally absent. The use of capillary endothelium in culture to assay angiogenesis for diagnostic and prognostic purposes is discussed. (13 Refs)

10. CELL CULTURE STUDIES ON NEUROFIBROMATOSIS (VON RECKLINGHAUSEN) I. COMPARATIVE GROWTH EXPERIMENTS WITH FIBROBLASTS AT HIGH AND LOW CONCENTRATIONS OF FETAL CALF SERUM.

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Abteilung Humangenetik, Universitat Ulm, P.O. Box 4066, D-7900 Ulm, W. Germany
Hum Genet; 58(2):188-193 1981

Growth experiments were performed to compare the serum requirement and final cell density of fibroblast cultures derived from patients (7 male, 4 female; 13-30 yr old) with neurofibromatosis (NF) with those from 13 healthy donors. Three kinds of primary cultures were set up from NF patients: small minced pieces of skin attached spontaneously to the surface of the culture flask, the skin being derived either from the upper arm or from the immediate vicinity of a neurofibroma; the analogous type of stem culture but with a minced piece of tumor; and finally, collagenase dissociated cells from tumors. The mean ratios of final to initial cell number did not differ

significantly between patients and controls, but a high capacity to grow in media containing 1% or 15% fetal calf serum (fcs) seemed to occur more frequently among NF strains than among control strains. In other experiments, more than three cell population doublings occurred 4x in NF strains and not at all in control strains. A significant difference between NF and control strains occurred only in the 15% fcs series. Higher post-confluent growth potentials occurred more frequently among NF strains than among control strains, although the difference of the frequency of high values reached statistical significance only in the 1% fcs series. In general faster growing strains exhibited lower protein content per cell and the slower higher amounts. The implications of the these findings are discussed. (29 Refs)

11. ACIDIC ISOFERRITINS (LEUKEMIA-ASSOCIATED INHIBITORY ACTIVITY) FAIL TO INHIBIT BLAST PROLIFERATION IN ACUTE MYELOGENOUS LEUKEMIA.

Taetle R
Univ. Hosp. H720, 225 Dickinson St., San Diego, CA, 92103
Blood; 58(3):653-657 1981

Cell-free extracts of bone marrow and blood cells from patients with leukemia contain an inhibitor of normal granulocyte/macrophage progenitor (CFU-GM) proliferation (leukemia-associated inhibitory activity, LIA) identified as acidic isoferritins. A comparison was made of the action of crude LIA Prepared from frozen-thawed leukemic blood and purified spleen ferritin from a patient with chronic myelogenous leukemia, on the proliferation of blast progenitors from patients with acute myelogenous leukemia (AML), and on the promyelocytic leukemia cell line, HL-60. Crude LIA showed no inhibition of blast progenitor or HL-60 proliferation at low concentrations, but inhibited the proliferation of CFU-GM. At higher concentrations, crude LIA inhibited both blast cells and CFU-GM. Purified spleen ferritin failed to inhibit blast progenitors or HL-60 cells at any concentration tested, but inhibited both 7-day and 14-day CFU-GM. Using the thymidine 'suicide' technique, the action of LIA was confirmed as being on CFU-GM in S-phase, but it failed to affect the proliferation of blast cells in S-phase. It is concluded that acidic isoferritins inhibit normal CFU-GM but not blast cells from patients with AML. Acidic isoferritins could confer a proliferative advantage of the leukemic clone over its normal counterparts. (Author abstract) (23 Refs)

12. SUPPRESSIVE EFFECT OF SUPERNATANT OF MIXED LYMPHOCYTE TUMOR CELL CULTURES (MLTC) ON THE GROWTH OF HUMAN GASTRIC CARCINOMA TRANSPLANTED IN NUDE MICE.

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Dept. Surgery, Sch. Medicine, Keio Univ., Shinjuku-ku, Tokyo 160, Japan
Nippon Geka Gakkai Zasshi; 82(4):305-315 1981

Human gastric carcinomas, KSt-1 and St-15, which had been maintained in nude mice, were used as human tumors, and the effect of human lymphocytes on the tumor growth was examined. The growth of St-15 in nude mice was temporarily inhibited by injection of human lymphocytes sensitized in MLTC but not by nonsensitized ones. Injection of supernatant of MLTC to tumor-bearing nude mice suppressed the tumor growth markedly. Spleen cells of nude mice injected with MLTC-supernatant showed significantly increased natural killer (NK) activity, as determined by 51Cr-release assay on YAC, and increased macrophage activity, as determined by inhibition of 3H-thymidine uptake by H-2 syngeneic P-815. These results indicate that human lymphocytes sensitized to tumor may recognize allogenic human lymphocyte antigen on tumor cells and temporarily inhibit the tumor growth, and that MLTC-supernatant activates NK cells as well as macrophages in tumor-bearing nude mice and these activated cells prevent the tumor growth. (39 Refs) (In JPN)

13. BIOLOGICAL ACTIVITY OF CHALONES ISOLATED FROM NORMAL AND REGENERATING LIVER.

Ketlinskii SA, Parfenova EV
Lab. Experimental Histology, Inst. Experimental Medicine, Leningrad, USSR
Biull Eksp Biol Med; 91(7):96-98 1981

To evaluate the mechanism of the disappearance of the biological activity of cha lones, random-bred hepatectomized rats were inoculated with chalones isolated from normal and regenerating liver. Chalones isolated 22 hr or 28 hr after hepatectomy failed to inhibit mitotic activity of the hepatocytes, but provided a 55% inhibition of DNA synthesis. It was suggested that the loss of chalone activity was associated with the changes in the competitive relationship between the inhibitors and stimulants of proliferation. (6 Refs) (In RUS)

14. EFFECT OF EPIDERMAL CHALONES ON CELL PROLIFERATION IN REGENERATING EPIDERMIS.

Ketlinskii SA
Lab. Experimental Histology, Inst. Experimental Medicine, Leningrad, USSR
Tsitologiia; 23(7):788-796 1981

The effect of epidermal chalones on proliferative activity of the regenerating epidermis was studied in C57Bl mice subjected to the plucking of hair from the back region. Chalone administration within the first 6 hr after the plucking decreased the fraction of labeled cells. Epidermal cells in G1 phase were less sensitive to chalones. Chalone administration 15 hr after the plucking prevented development of hyperplasia of the epithelial layer. Repeated administration of chalones prior to the plucking inhibited proliferative activity for 25 hr. (30 Refs) (In RUS)

15. FACTORS STIMULATING AND INHIBITING TUMOR GROWTH IN CARCINOGENESIS.

Kochergina NI, Kosova IP
Res. Lab. Experimental Immunobiology, Moscow, USSR
Usp Sovrem Biol; 91(3):409-418 1981

Literature data on tumor factors that can stimulate or inhibit proliferation of normal or neoplastic cells are reviewed. Factors isolated from murine sarcoma 180, spontaneous and explanted murine mammary gland carcinomas, tissue-specific inhibitors of cell divisions (chalones), and polysomal RNA from Rous sarcoma cells were found to regulate the neoplastic growth and to affect tumor transplantability. Tumor factors were shown to inhibit the antibody formation. The sera of cancer patients was shown to contain factors that inhibited lymphocyte cytotoxicity. In response to stimulation with tumor antigens, T lymphocytes formed the clone of killer cells. The degree of lymphocyte transformation depended upon the ratio of lymphocytes and tumor antigen. Tumor factors were found to inhibit not only the antitumor but also the transplacental immunity. Stimulation of tumor antigenicity can stimulate the immune system of tumor-bearing organism and thus can have an immunotherapeutic effect. (108 Refs) (In RUS)

HORMONES/ STEROIDS/ PROSTAGLANDINS AS GROWTH EFFECTORS

16. SPECIFIC TRIIODOTHYRONINE BINDING BY TUMOR CELLS AND SPLEEN CELLS IN A THYROID HORMONE DEPENDENT MOUSE TUMOR SYSTEM.

Gupta MK, Chiang T, Deodhar SD
Dept. Immunopathology, Cleveland Clinic Foundation, 9500 Euclid Ave., Cleveland, OH, 44104
Eur J Cancer Clin Oncol; 17(7):819-823 1981

We have reported thyroid hormone dependency of syngeneic mouse tumor (fibrosarcoma T241 in C57Bl/6J mice) for growth and spread. To study further the mechanisms of thyroid hormone action we assayed tumor cells and spleen cells for thyroid hormone receptors. Cells were incubated at 37 C for 1 hr with [125I]triiodothyronine with and without unlabeled T3. Competitive inhibition of [125I]T3 bound to spleen cells was demonstrated by increasing the amount of unlabeled T3 and T4. Similar competitive inhibition of binding was minimal for intact tumor cells. Saturable binding sites in nuclei were demonstrated in all these cell populations. A Scatchard analysis of nuclei fraction from these cells showed equilibrium dissociation constant (Kd) of 0.56 x 10(-9) M for normal spleen cells and tumor cells and 1.4 x 10(-9) M for tumor-bearing mice spleen cells. The estimated max binding capacity for nuclei of these cells was 26 fmol/10 x 10(6) cells for normal spleen cells, 46 fmol/10 x 10(6) cells for tumor-bearing mice spleen cells and 45 fmol/10 x 10(6) cells for tumor cells. Our results established the presence of T3 receptors in nuclei of tumor cells as well as spleen cells and suggests the direct metabolic effect of thyroid hormone as a possible mechanism for thyroid hormone dependency of this tumor. (Author abstract) (21 Refs)

17. BETA2 RECEPTOR-MEDIATED STIMULATION OF FRIEND ERYTHROLEUKEMIA CELL GROWTH BY THYROID HORMONES.

Gauwerky CE, Bersch NL, Golde DW
Div. Hematology-Oncology, Dept. Medicine, Univ. California Los Angeles Sch. Medicine, Los Angeles, CA, 90024
Exp Hematol; 9(8):821-828 1981

Thyroid hormones are known to enhance normal erythroid colony growth (CFUE) and this enhancement depends on a functional beta2-adrenergic receptor mechanism. We investigated the response of Friend cells to thyroid hormones, catecholamines, and other compounds influencing cellular cAMP activity. The thyroid hormones L-T3, L-T4, and 'reverse T3' stimulated erythroleukemia colony growth in a serum-substituted methylcellulose culture system with peak activity at 10(-7) M. Various beta-adrenergic compounds enhanced Friend leukemia colony growth; however, the alpha-adrenergic agonist phenylephrine was inactive. Dibutyl cyclic AMP and the phosphodiesterase inhibitor theophylline also enhanced Friend leukemia colony formation. Adrenergic antagonists with beta2 specificity abrogated the stimulatory effect of L-T3, L-T4, and of 'reverse T3' at equimolar concentrations. These experiments demonstrate that thyroid hormones, beta-adrenergic agonists, the phosphodiesterase inhibitor theophylline, and dbcAMP have a direct effect on the proliferation of Friend erythroleukemia cells. We conclude that thyroid hormones' action requires a functioning beta2-adrenergic receptor mechanism. Thyroid hormones directly modulate the growth of neoplastic erythroid cells in a manner consistent with their effects on normal erythropoiesis. (Author abstract) (31 Refs)

18. INHIBITION OF CLONOGENIC GROWTH OF MELANOMA CELLS BY COMBINATION OF MELANOCYTE STIMULATING HORMONE AND THEOPHYLLINE.

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Sidney Farber Cancer Inst., 44 Binney St., Boston, MA, 02115
J Invest Dermatol; 77(3):253-255 1981

The combination of MSH and theophylline is synergistic in inhibiting the growth as well as stimulating the differentiation of pigmented melanoma cells. In order to determine if a permanent alteration in the proliferative potential of these cells was achieved, we examined the effect of MSH and theophylline alone and in combination upon the plating efficiency and tumorigenicity of S-91 Cloudman melanoma cells. Following treatment with the combination of MSH and theophylline, the cells became deeply pigmented and had their colony forming ability reduced to 28% of control. Neither MSH or theophylline alone, although effective in reducing the growth rate of the cells, caused a significant reduction in clonogenic growth. When cells were pretreated with MSH and theophylline alone or in combination and reinjected into mice, the combination of MSH and theophylline caused a significant reduction in percentage of animals developing tumor (reduced to 14% of control). Cells treated with either MSH or theophylline alone were similar in their tumorigenic potential to control cells. These effects were selective since the nonmelanocytic control cells, mouse fibroblast L929 and Chinese hamster ovary when treated similarly did not exhibit any alteration in their clonogenic potential. The treatment of responsive cells with the combination of MSH and theophylline is capable of causing permanent decrease in the proliferative potential of murine melanoma cells and may have relevance to the treatment of this disease in man. (Author abstract) (12 Refs)

19. INTERACTION OF VASOACTIVE INTESTINAL PEPTIDE WITH A CELL LINE (HELA) DERIVED FROM HUMAN CARCINOMA OF THE CERVIX: BINDING TO SPECIFIC SITES AND STIMULATION OF ADENYLATE CYCLASE.

Prieto JC, Guerrero JM, De Miguel C, Goberna R
Dept. Biochemistry, Sch. Medicine, Sevilla, Spain
Mol Cell Biochem; 37(3):167-176 1981

The binding of vasoactive intestinal peptide (VIP) and its effect on cyclic AMP production were assessed in HeLa cells. The binding of [125I]VIP is a moderately rapid process, reversible, saturable, specific and dependent on temperature. Virtually no inactivation of the peptide is observed after 2 hr of exposure to the cells. At 15 C, the binding data obtained at steady state are compatible with the existence of two classes of binding sites: a first class with a Kd of 2.4 nM and low binding capacity (1.5 x 10(5) sites/cell) and a second class with a Kd of 100 nM and a high binding capacity (4.9 x 10(6) sites/ cell). Secretin is eight times less potent than VIP in competing with 125I VIP but glucagon, insulin and somatostatin are inactive. VIP-induced stimulation of cyclic AMP production depends on time and temperature and is potentiated by a phosphodiesterase inhibitor. A concentration of VIP as low as 10(-10) M is able to stimulate adenylate cyclase. Half-max stimulation is observed at 10(-9) M and max stimulation (4x above basal levels) at 10(-8) M VIP. Secretin is an agonist of VIP but exhibits a 1,000x lower potency with respect to adenylate cyclase activation. Glucagon, insulin and somatostatin do not show any effect. The presence of high-affinity binding sites and the high sensitivity and specificity of adenylate cyclase for VIP in HeLa

cells provide a good model to study the role of this peptide on cell proliferation and differentiation. (Author abstract) (38 Refs)

20. PROSTAGLANDINS AND MYELOPOESIS: EFFECT OF PROSTAGLANDIN E1 ON NORMAL AND CHRONIC MYELOID LEUKEMIA COLONY FORMING CELLS (CFU-GM) SUBPOPULATIONS.

Aglietta M, Piacibello W, Gavosto F
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Cell Biol Int Rep; 5(9):836 1981

When normal and chronic myeloid leukemia (CML) cells were incubated in the presence of increasing concentrations of prostaglandin E1 (PGE1), a significant ($p. = 0.05$) difference was seen in the responsiveness of the cells to PGE1 after 7-8 days of incubation: the colony forming cells (CFU-GM) among the normal cells were inhibited by very low (10(-10) M) concentrations of PGE1 whereas CML CFU-GM were insensitive to PGE1 concentrations up to 10(-7) M. The difference was also seen after 14 days of PGE1 incubation. The data suggest mechanisms responsible for the *uncontrolled proliferation* of CML cells. (4 Refs)

CYCLIC NUCLEOTIDE/ POLYAMINES/ NUCLEOTIDES AS GROWTH EFFECTORS

21. EFFECTS OF CYCLIC-NUCLEOTIDE DERIVATIVES ON THE GROWTH OF HUMAN COLONIC CARCINOMA XENOGRAFTS AND ON CELL PRODUCTION IN THE RAT COLONIC CRYPT EPITHELIUM.

Tutton PJ, Barkla DH
Dept. Anatomy, Faculty Medicine, Monash Univ., Clayton, Victoria 3168, Australia
Br J Cancer; 44(2):182-188 1981

Previous studies have shown that various amine hormones are able to influence the growth rate of human colorectal carcinomas propagated as xenografts in immune-deprived mice, and it is now well known that the effects of many amine and other hormones are mediated by cyclic nucleotides, acting as second messengers within cells. In the present study the influence of various derivatives of cyclic AMP and cyclic guanosine monophosphate on the growth of two different lines of colorectal cancer growing in immune-deprived mice, and on the cell production rate in the colonic crypt epithelium of the rat, was assesssed. Growth of each tumor line, as well as crypt-cell production, was suppressed by treatment with N6O2' dibutyryl and N6 monobutyryl derivatives of cyclic AMP. Dibutyryl cyclic guanosine monophosphate, on the other hand, was found to promote the growth of Tumor HXK4 and to promote crypt cell production, but to have no significant effect on Tumor HXM2. (Author abstract) (35 Refs)

22. DIFFERENTIAL SENSITIVITY OF NORMAL AND CHEMICALLY TRANSFORMED EPITHELIAL CELLS TO CHOLERA TOXIN.

Niles RM, Loewy B, Krah D
Dept. Biochemistry, Boston Univ. Sch. Medicine, Boston, MA, 02118
Cancer Res; 41(10):4075-4079 1981

We have been studying the regulation of growth by cyclic AMP and other factors in untransformed (K16) and chemically transformed (W8) rat liver epithelial cells. Initially, we found that 8-bromocyclic adenosine 3':5'-monophosphate was a more potent inhibitor of cell replication in K16 than in W8 cells. In addition, the phosphodiesterase inhibitor 1-methyl-3-isobutylxanthine (MIX) caused marked growth inhibition in K16 but not in W8 cells. Through the use of cholera toxin (CT) with or without MIX, we elevated intracellular cyclic AMP levels in a quantifiable fashion. With CT alone or combined with MIX, we observed a dose-dependent morphological change in W8 cells, which consisted of extensive 'process' formation. K16 morphology was not altered at any concentration of CT +- MIX tested. K16 cell growth was only marginally inhibited by CT alone, but markedly inhibited by CT plus MIX. W8 cell growth was moderately inhibited by CT alone or combined with MIX. Analysis of cyclic AMP levels revealed that, at all concentrations of CT +- MIX and at all time periods tested, W8 cells produced significantly more cyclic AMP than K16 cells. It appears that morphological changes and growth inhibition are not necessarily linked and that MIX may inhibit K16 cell replication by means other than its ability to increase intracellular cyclic AMP levels. (Author abstract) (23 Refs)

23. GROWTH ARREST AND MORPHOLOGICAL CHANGE OF HUMAN BREAST CANCER CELLS BY DIBUTYRYL CYCLIC AMP AND L-ARGININE.

Cho-Chupg YS, Clair T, Bodwin JS, Berghoffer B
Cellular Biochemistry Section, Lab. Pathophysiology, NCI, Bethesda, MD, 20205
Science; 214(4516):77-79 1981

The growth in vitro of human breast cancer cells, line MCF-7, was inhibited by a daily supplement of L-arginine (1 mg per ml). Arginine acted synergistically with dibutyryl AMP (cyclic AMP) (10(-6) M) to enhance the growth inhibitory effect: the cell replication ceased completely within 2 days after treatment. The growth arrest accompanied a change in cell morphology and was preceded by increases in the cellular concentration of cyclic AMP, adenylate cyclase, and Type II cyclic AMP-dependent protein kinase activities as well as a decrease of estrogen binding activity. The results suggest that growth of human breast cancer cells is subject to cyclic AMP-mediated regulation and that arginine may play a specific role in this process. (Author abstract) (22 Refs)

COFACTORS/ NUTRITIONAL GROWTH FACTORS

24. 1,25-DIHYDROXYVITAMIN D3 SPECIFICALLY BINDS TO A HUMAN BREAST CANCER CELL LINE (T47D) AND STIMULATES GROWTH.

Freake HC, Marcocci C, Iwasaki J, MacIntyre I
Endocrine Unit, Royal Postgraduate Medical Sch., Du Cane Road, London, W12 OHS, England
Biochem Biophys Res Commun; 101(4):1131-1138 1981

The binding of the active vitamin D3 metabolite 1,25-dihydroxyvitamin D3 (DHD) to receptors on T47D breast carcinoma cells in vitro was studied. A specific receptor with a dissociation constant of 1.1 x 10(-10), a sucrose gradient sedimentation coefficient of 3.7S and a frequency of 15,000 sites/cell was identified. Growth of T47D cells was enhanced by DHD in a dose-dependent manner at physiological doses up to 0.8 nanoM. At higher doses the effect was eliminated. Growth was

also enhanced by estradiol. Metabolites of vitamin D3 showing affinity for the binding sites, in decreasing order of affinity, were the 1,25-dihydroxy, 1,24,25-trihydroxy, 25 hydroxy, and 24,25-dihydroxy derivatives. The new metabolite 1beta,25-dihydroxyvitamin D3 had the same affinity as the 24,25 dihydroxy metabolite. The binding site was retained in culture cell lines from the parental tissue. The in vivo significance of vitamin D3 effects on breast cancer cells is unknown. (15 Refs)

25. VITAMIN K3 (MENADIONE) INHIBITS THE GROWTH OF MAMMALIAN TUMOR CELLS IN CULTURE.

Prasad KN, Edwards-Prasad J, Sakamoto A
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Life Sci; 29(13):1387-1392 1981

The effects of vitamin K on the morphology and the growth of mouse neuroblastoma (P2), mouse melanoma (B-16) and rat glioma (C-6) cells in culture were studied. Vitamin K3 inhibited the growth (due to cell death and partial or complete inhibition of cell division) of all three cell types without causing any morphological differentiation. Vitamin K3 was more effective than vitamin K1. Neuroblastoma cells were more sensitive to vitamin K3 than were melanoma or glioma cells. Glioma cells did not grow in hormone-supplemented serum-free medium; however, both neuroblastoma and melanoma cells grew to a level 70-80% of that found in serum-supplemented medium. Neuroblastoma cells and melanoma cells cultured in serum-free medium exhibited a 2-3 fold higher sensitivity to vitamin K3 than those cultured in serum-supplemented medium. This suggests that serum factors attenuate the growth inhibitory effect of vitamin K3 on tumor cells in culture, probably by reducing the availability of this vitamin to the cells. Neuroblastoma cells were more sensitive to vitamin K3 than were melanoma cells even when they were treated in serum-free medium. The fact that uM concentrations of vitamin K3 inhibit the growth of tumor cells in culture suggests that this vitamin may be a potentially useful anticancer agent. (Author abstract) (11 Refs)

26. EFFECT OF DIETARY AMINO ACIDS ON TUMOUR GROWTH AND CELL-MEDIATED IMMUNE RESPONSES.

Bounous G, Sadarangani C, Pang KC, Kongshavn PA
Centre Hospitalier Universitaire, Sherbrooke PQ J1H 5N4, Canada
Clin Invest Med; 4(2):109-115 1981

The effects of various diets were studied in C3H/NCrlBR, C57Bl/6, and (AxC57Bl/6)F1 mice. Diet 1, the standard defined-formula amino acid diet, contained about the same amino acid distribution as casein. Diet 2 contained reduced amounts of l-methionine, l-phenylalanine, and l-tyrosine, and no cystine. Diet 3 contained almost double the amount of amino acids found in diet 1. The Puria mouse chow used contained approx 18% protein. Diet 1 supported normal growth in C3H/NCrlBR mice. Diet 3 and Purina did not produce more body growth than diet 1. Diet 2 produced impaired body growth and reduced spleen wt and WBC counts. Skin grafts were rejected sooner on mice fed diet 2 than on mice fed diet 1. Mice fed diet 2 had higher resistance to Listeria monocytogenes infection than those fed diet 1 or Purina. Diet did not affect the time of appearance of palpable fibrosarcomas. Palpable mammary adenocarcinomas appeared soonest in mice fed diet 3 or Purina. Diets 1 and 2 had about the same inhibitory effect on fibrosarcoma growth. Diet 2 had a greater inhibitory effect on the growth of mammary adenocarcinomas. Tumor growth showed a direct dose-response relationship to amino acid content of the diet. Graft-vs-host reactivity was not affected by diet. Evidently reduction of amino acid inhibits tumor growth, while excessive amino acids promotes tumor growth. This suggests that current concepts of 'good nutrition' promoting immune defenses may be in error. (26 Refs)

EXOGENOUS AGENTS AFFECTING DIFFERENTIATION

27. REGULATION OF GENE EXPRESSION BY TUMOR PROMOTERS. II. CONTROL OF CELL SHAPE AND DEVELOPMENTAL PROGRAMS FOR MACROPHAGES AND GRANULOCYTES IN HUMAN MYELOID LEUKEMIC CELLS.

Liebermann D, Hoffman-Liebermann B, Sachs L
Dept. Genetics, Weizmann Inst. Science, Rehovoth 76100, Israel
Int J Cancer; 28(3):285-291 1981

Regulation of the developmental programs for macrophages and granulocytes has been analyzed, using two-dimensional gel electrophoresis of the cytoplasmic protein changes, in a human myeloid leukemic cell line (HL60) that can be induced to differentiate to macrophages by the macrophage and granulocyte-inducing (MGI) protein and the tumor promoter 12-O-tetradecanoyl-phorbol-13-acetate (TPA), and to granulocytes by dimethylsulfoxide (DMSO). Studies on the protein changes induced by the differen t inducers showed a similar developmental program for macrophage differentiation induced by MGI or TPA, which differed from the beginning from the granulocyte program induced by DMSO. Comparison with normal cells from human peripheral blood has shown that the developmental programs induced in the HL60 cells are relevant to the normal programs of differentiation for these two cell types. Unlike MGI and DMSO, TPA induces rapid attachment of the HL60 cells to the surface of the Petri dish. Combined treatment with TPA and DMSO showed cell attachment, extensive spreading of the cells, the regulation of specific proteins and expression of the macrophage program. The addition of TPA to cells induced for the granulocyte program by DMSO resulted in cell attachment and spreading and a switch-over from the granulocyte to the macrophage program. The results indicate that cells in suspension can express either the macrophage or granulocyte program depending on the inducer, and that changes in cell shape associated with cell attachment induced by TPA can regulate specific proteins and restrict the developmental program to macrophages. It is suggested that the in vivo environment of cells in relation to the possibilities for cell adhesion, which can be regulated by tumor promotors, may play a major role in determining the developmental program of myeloid and other cell types. (Author abstract) (41 Refs)

28. DIFFERENTIATION OF MOUSE MYELOID LEUKEMIA CELLS INDUCED BY 1ALPHA,25-DIHYDROXYVITAMIN D3.

Abe E, Miyaura C, Sakagami H, Takeda M, Konno K, Yamazaki T, Yoshiki S, Suda T
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Proc Natl Acad Sci USA; 78(8):4990-4994 1981

Mouse myeloid leukemia cells can be induced to differentiate into macrophages in vitro by 1alpha,25-dihydroxyvitamin D3, the active form of vitamin D3. The minimal concentration of 1alpha,25-dihydroxyvitamin D3 to induce the cell differenti-

ation was 0.12 nM. The degree of cell differentiation in various markers induced by 12 nM 1alpha,25-dihydroxyvitamin D3 was nearly equivalent to that induced by 1 uM dexamethasone, the most potent known stimulator. Among several markers of the differentiation by 1alpha,25-dihydroxyvitamin D3, phagocytic activity was induced within 24 hr, and this was followed by induction of lysozyme and locomotive activities. Similar changes were also induced by 0.01-1.00 uM 1alpha-hydroxyvitamin D3. 25-Hydroxyvitamin D3 and 24R,25-dihydroxyvitamin D3 showed only weak inducing activity. These results suggest the possibility that, in addition to its well-known biological activities in enhancing intestinal calcium transport and bone mineral mobilization, 1alpha,25-dihydroxyvitamin D3 is involved in the differentiation of bone marrow cells. (Author abstract) (27 Refs)

29. CLOSE ASSOCIATION OF MOUSE MYELOID LEUKEMIA (M1) CELL DIFFERENTIATION WITH CESSATION OF DNA SYNTHESIS.

Sakagami H, Unten S, Konno K
Dept. Biochemistry, Sch. Medcine, Showa Univ., 1-5-8 Hatanodai, Shinagawa-ku, Tokyo 142, Japan
Cell Struct Funct; 6(2):147-157 1981

Experiments were carried out to determine whether growth retardation in terminally differentiated cells in culture is due to the artefactual production of dead cells or to some intrinsic phenomenon in the cells. Mouse myeloid leukemia M1 cells that had been induced to differentiate in vitro by 10(-6) M *dexamethasone* (DM) treatment for varying time periods (0-65 hr) were assayed for DNA synthesis by measuring the incorporation of radioactive thymidine into acid-insoluble material. DNA synthesis began to decline after 10 hr of DM treatment and reached one-half the control level after 65 hr; both DM-treated and control cells incorporated approx 20% of the added thymidine initially added to the culture medium and nearly all of this radioactivity was recovered from the acid-soluble fraction, indicating that there is no significant catabolization and reutilization of incorporated label in nonDNA fractions over the time of the experiment; autoradiographs showed that 96.8-98.6% of the label resided in the nucleus. The administration of greater than 10(-4) M arabinofuranosylcytosine inhibited 95% of the thymidine incorporation. The DM-treated cells were fractionated into nonadherent and adherent subpopulations and the adherent cells were gradient-fractionated into further subpopulations based on their density (that reflects the ratio of nucleus to cell size [N/C ratio]). In general, DNA synthesis declined as cell density declined; autoradiographic analysis revealed that this decrease was due to the accumulation of unlabeled cells (cells that cannot carry out DNA replication) with a much lower N/C ratio than the labeled cells. When the distribution of DNA content was measured by flow microfluorometry, the DM-treated cell population contained approx 50% fewer S-phase cells than the untreated population. These results indicate that M1 differentiation is closely coupled with the cessation of DNA synthesis. (19 Refs)

30. INDUCTION OF DIFFERENTIATION OF LEUKEMIA CELLS IN VITRO BY N-SUBSTITUTED AMIDES, LACTAMS, AND 2-PYRIDONES.

Li C, Schwartz EL, Mella SL, Rittmann LS, Sartorelli AC
Dept. Pharmcology, Comprehensive Cancer Center, Yale Univ. Sch. Medicine, New Haven, CT, 06510
J Med Chem; 24(9):1092-1094 1981

N-Substituted amides, lactams, and 2-pyridones were examined for their ability to induce leukemic cell differentiation in vitro. These substituted amides were effective inducers of the differentiation of murine erythroleukemia (Friend) cells, as judged by the cellular accumulation of Hb. N- Substitution increases the effectiveness of all classes of agents as inducers of maturation as judged by an increase in potency. N-Cyclohexylacetamide appeared to be a particularly effective compound, causing 92% of the population to synthesize Hb at a concentration which was not inhibitory to cell replication. In the lactam series, an increase in ring size was paralleled by an increase in the potency of unsubstituted compounds, but ring size had less effect on N-substituted lactams. Increasing the chain length of the N-alkyl substituent had little additional effect on a series of lactam and 2-pyridone derivatives; however, some decrease in maximal induction occurred with butyl- and hexyl-substituted 2-pyridones. N-Isopropyl-2-pyridone, selected for further study, was as good an inducer of the HL60 human promyelocytic leukemia as it was of the Friend murine erythroleukemia. (Author abstract) (16 Refs)

31. MODIFICATION OF LIPOPHILIC PROTEINS IN FRIEND ERYTHROLEUKEMIA CELLS DURING THEIR DIFFERENTIATION.

Semmel M, Delain E
Groupe 8 du C.N.R.S., Institut Gustave-Roussy, 94800 Villejuif, France
Mol Biol Rep; 7(4):247-252 1981

Friend mouse erythroleukemia clones 745 A, F4-1, TFP-10, and TFA-11 were treated with dimethylsulfoxide (DMSO) to induce differentiation. Cells were labeled and analyzed for Hb, reverse transcriptase, and lipophilic membrane proteins. Fixed thin sections were stained and examined by electron microscopy. Electrophoretic profile of extracted lipophilic proteins showed an increase in the proportion of 9.5 kilodalton (kD) material in differentiated cells. Benzidine testing indicated that the increase preceded Hb synthesis by at least 1 day. DMSO treatment elevated the amount of reverse transcriptase only in the supernatant of TFA-11 cells. Because the 9.5 D protein increase occurred in line F4-1 (which does not produce virus) but not in TFA-11, it apparently was not associated with virus production. Cells treated with the inducers hexamethylenebisacetamide, aminonucleoside of pyromycine, or butyric acid exhibited an increase comparable to that seen with DMSO, but in combination with antagonists 1,2-O-tetradecanoyl-phorbol-13-acetate or inosine the increase was less marked. The increase in 9.5 kD material thus appeared unlikely to result from an effect of the inducers unrelated to differentiation; it is thought related to differentiation and due either to increased biosynthesis or to a structural modification impairing the protein's capacity to polymerize or favoring extractability. (26 Refs)

32. CYCLIC UREA AND THIOUREA DERIVATIVES AS INDUCERS OF MURINE ERYTHROLEUKEMIA DIFFERENTIATION.

Li C, Mella SL, Sartorelli AC
Dept. Pharmacology, Comprehensive Cancer Center, Yale Univ. Sch. Medicine, New Haven, CT, 06510
J Med Chem; 24(9):1089-1092 1981

A series of derivatives of tetramethylurea, a known inducer of the differentiation of Friend erythroleukemia cells, has been synthesized and tested for its capacity to induce erythroid maturation, as measured by the synthesis of Hb. Cyclic urea and thiourea derivatives consisting of five-, six-, and seven-membered ring systems containing N-alkyl substituents were pre-

pared. Most of these agents were relatively effective inducers of differentiation, with N-alkyl substitution appearing to be essential for max response. The most potent agents developed were N,N'-dimethyl cyclic ureas. Exposure to concentrations of 2 to 4 mM of these derivatives resulted in more than 90% of the cell population achieving a differentiated state. Under these conditions, the parent compound, tetramethylurea, was slightly less efficacious, causing differentiation of only 68% of the population at its max effective level of 4 mM. (Author abstract) (32 Refs)

OTHER RELATED STUDIES

33. THE ROLE OF INTERLEUKIN-2 (IL-2) IN THE DIFFERENTIATION OF CYTOTOXIC T CELLS: THE EFFECT OF MONOCLONAL ANTI-IL-2 ANTIBODY AND ABSORPTION WITH IL-2 DEPENDENT T CELL LINES.

Kern DE, Gillis S, Oksda M, Henney CS
Basic Immunology Program, Fred Hutchinson Cancer Res. Center, 1124 Columbia St., Seattle, WA, 98104
J Immunol; 127(4):1323-1328 1981

A variety of approaches, including the use of a monoclonal antibody, have indicated that the lymphokine interleukin 2 is a necessary and sufficient mediator for initiating cytotoxic T cell differentiation in vitro. Mitogen (Con A, PHA) stimulation of spleen cells and K/D or I region stimulation in primary MLC all led to IL-2 production, as measured by the growth of an IL-2 dependent T cell clone, and to the production of a 'helper' factor necessary for cytotoxic T lymphocyte (CTL) differentiation against metabolically inactivated stimulator spleen cells. Several lines of evidence suggested that the IL-2 activity and CTL 'helper' activity were due to a single molecular entity. These included the observations that: (i) there was a linear relationship (R = 0.99) between IL-2 activity and relative CTL 'helper' activity regardless of the preparation used (purified IL-2; MLC, or mitogen-induced supernatants). (ii) Absorption of MLC supernatants with T cell 'blasts' or with IL-2 dependent T cell lines concomitantly removed both IL-2 and CTL 'helper' activities, (iii) IL-2 activity purified by isoelectric focusing, or by polyacrylamide gel electrophoresis, displayed CTL 'helper' activity. (iv) Both CTL 'helper' and IL-2 activities were neutralized by a monoclonal antibody directed against IL-2. (Author abstract) (21 Refs)

34. PLURIPOTENT, ERYTHROCYTIC AND GRANULOCYTIC HEMOPOIETIC PRECURSORS IN CHRONIC GRANULOCYTIC LEUKEMIA.

Hara H, Kai S, Fushimi M, Taniwaki S, Ifuku H, Okamoto T, Obe Y, Fujita S, Noguc hi K, Kanamaru A, Nagai K, Inada E
Div. Blood Transfusion, Hyogo Coll. Medicine, 1-1, Mukogawa-cho, Nishinomiya, Hyogo 663, Japan
Exp Hematol; 9(8):871-877 1981

Using methylcellulose culture techniques, we investigated the behavior of committed and pluripotent hemopoietic precursors (CFUMIX) in marrow cells and blood from patients with chronic granulocytic leukemia (CGL) in the chronic phase whose marrow cells showed the presence of Ph1 chromosomes. The relative concentrations of the marrow precursors in CGL were nearly equal to those in control patients. The mean nucleated cell count in marrow fluid from the patients, however, was 6.3x that of control values. As a result, the mean concentrations per unit vol of the hemopoietic precursors in CGL were significantly higher than those in control patients. Furthermore, the presence of erythropoietin (Ep) independent BFUE giving rise to erythropoietic bursts in the medium without the addition of Ep was demonstrable in marrow cells and blood. In the peripheral blood, the increment in numbers of CFUC, CFUE, BFUE, and CFUMIX in accordance with the WBC count was observed. The present results are consistent with the theory that the clonal expansion in CGL occurs at the level of pluripotent hemopoietic precursors and suggest that CFUMIX in CGL can differentiate into CFUE along the erythropoietic pathway and that a regulatory defect in CGL is present in both granulocytic cells and erythrocytic cells. (Author abstract) (26 Refs)

35. RECOGNITION OF MOPC-460 VARIABLE REGION DETERMINANTS BY POLYCLONALLY DISTRIBUTED TRIGGERING RECEPTORS ON B LYMPHOCYTES.

Primi D, Juy D, Le Guern C, Sanchez P, Cazenave PA
Unite d'Immunochimie Analytique, Institut Pasteur, 28 rue du Dr. Roux, 75724 Paris Cedex 15, France
J Immunol; 127(4):1714-1718 1981

Purified M460 protein-induced untreated and anti-Thy 1.2 and complement-treated murine spleen cells to proliferate and differentiate in vitro. Fc receptor complexing was not responsible for the phenomenon, since M460 Fab fragments retained the B cell triggering activity. Activation was inhibitable by the addition of DNP-glycine to cultures and could also be detected by using spleen cells from various strains of mice, including the C3H/HeJ, which is genetically nonresponding to LPS. Hybrid molecules were constructed between the M460 proteins and the products of two myelomas that do not activate B cells, namely, X25 and X24. The only hybrid molecules that retained triggering activity were (L460-HX25)2. Interestingly, the same molecule was also the only one recognized by an anti-M460 monoclonal antibody (F6(51)) that has been previously shown to induce B cells to proliferate and differentiate. The data suggest the existence on B lymphocytes of a network of polyclonally distributed mitogen receptors that share determinants cr oss-reacting with Ig idiotypes. (Author abstract) (21 Refs)

36. METABOLIC HOST REACTION IN RESPONSE TO THE PROLIFERATION OF NONMALIGNANT CELLS VERSUS MALIGNANT CELLS IN VIVO.

Karlberg I, Edstrom S, Ekman L, Johansson S, Schersten T, Lundholm K
(c/o Lundholm) Dept. Surgery I, Sahlgrenska Sjukhuset, Univ. Goteborg, S-413 45 Goteborg, Sweden
Cancer Res; 41(10):4154-4161 1981

This study was aimed at elucidating whether proliferation of malignant cells is a prerequisite for the development of the multiform relationship between a malignant tumor and its host. A methylcholanthrene-induced sarcoma (MCG 101) transplanted sc in nongrowing wt-stable mice (C57BL/6J) was used as the tumor model. Injection ip of Corynebacterium parvum vaccine in C57BL/6J mice stimulated normal cells to exponential cell growth. This experimental model was considered suitable for elucidating the metabolic host response to proliferation of nonmalignant cells. C parvum-stimulated animals showed an exponential growth rate of the spleen and liver leading to a more than doubled tissue mass over 9 days. This was compared with the exponential growth rate of the tumor over 10 to 12 days in tumor-bearing mice. Tissues other than the tumor and the spleen showed unchanged or decreased dry wt in tumor mice, indicating in terms of wt a proliferation mainly of tumor

tissue, which was confirmed by histological examination and biochemical analyses in various tissues. Metabolic balance studies, body composition, evaluation of hepatic and muscle protien synthesis, as well as energy metabolism and measurement of the plasma concentration of various substances, were performed in C parvum-stimulated animals. All together 29 different parameters were selected for measurement in C parvum-stimulated animals. These parameters which reflected the overall and intermediary metabolism were compared with the results obtained in previous studies in tumor-bearing mice. Twenty-one of the 29 parameters showed the same pattern of alteration in C parvum-stimulated animals as in tumor-bearing animals. Frequency analyses showed no significant difference between the metabolic host reactions in the C. parvum-stimulated animals compared with the 'tumor-stimulated' animals, suggesting a similar response in these models. These experimental models seem therefore suitable for comparison of, and may be useful for mechanistic analyses of, the metabolic host reaction in response to proliferation of malignant versus non-malignant cells. We conclude that several metabolic host reactions found in tumor-bearing animals may be directly coupled to cell proliferation per se but not necessarily to the proliferation of malignant cells. (Author abstract) (44 Refs)

37. THE EFFECT OF LOW-MOLECULAR-WEIGHT INHIBITORS ON THE GROWTH IN VITRO OF A HUMAN PANCREATIC ADENOCARCINOMA CELL LINE.

Grant AG, Grant DA
Dept. Surgery, St. George's Hosp. Medical Sch., Cranmer Terrace, London SW17 0RE, England
Biochem Soc Trans; 9(1):134 1981

Inhibition of growth in an in vitro human pancreatic exocrine adenocarcinoma cell line was studied. Low mol wt proteinase inhibitors included antipain and leupeptin (active against trypsin-like enzymes), pepstatin (active against pepsin-like enzymes), and elastatinal (active against elastase-like enzymes). Growth was decreased 50% by 5-25 uM leupeptin or antipain. Cell numbers were reduced by 50 uM leupeptin to 35% and by 50 uM antipain to 25% of control values. Pepstatin and elastinal had no effect. [3H]-thymidine labeling occurred in 63% of controls within 24 hr. Doubling time was delayed 24 hr by 5 uM leupeptin. Antipain (5 uM) had no effect on cell division rate, but decreased the number of cells reaching interphase. Reseeding of cells in medium without inhibitors resulted in return to normal growth rate, suggesting that the inhibition was reversible. No cell toxicity was noted for 5-25 uM antipain or leupeptin. In tests to quantify the amount of inhibitor in culture medium by measuring inhibition of trypsin activity in cell-free medium, trypsin hydrolysis was reduced to a constant value regardless of whether the medium was assayed before, during, or after growth studies. This indicated that the inhibitors were not taken up by cells, but acted extracellularly. Results suggest that in vivo use of proteinase inhibitors for treatment of tumors would require long-term administration. (6 Refs)

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CELL AND SERUM DERIVED GROWTH EFFECTORS

1. EFFECT OF EPIDERMAL GROWTH FACTOR ON PROLIFERATION OF HUMAN TUMOR CELLS IN SOFT AGAR.

Hamburger AW, White CP, Brown RW
Cell Culture Dept., Americn Type Culture Collection, 12301 Parklawn Drive, Rockville, MD, 20852
JNCI; 67(4):825-830 1981

The effect of epidermal growth factor (EGF) and fibroblast feeder layers on the proliferation of human tumor cells in soft agar was examined. The addition of EGF to medium support-

HE 20.3172/2

ing growth of tumor cells significantly increased (p less than or equal to 0.02) the number of colonies grown from the cells of 36 of 58 patients (62%) with a variety of neoplasms. The increase in the number of colonies was de pendent on the concentration of EGF and was max at a concentration of 50 ng EGF/ml. The addition of glucocorticoids did not potentiate the effect of EGF. Lethally irradiated fibroblast feeder layers alone only slightly increased the number of colonies. However, the addition of fibroblasts to cultures significantly increased the mitogenic effect of EGF. The results suggest that a proportion of epithelial-derived tumors retain responsiveness in vitro to physiologic growth regulators such as EGF. (Author abstract) (28 Refs)

2. THE EFFECTS OF SODIUM BUTYRATE ON EPIDERMAL GROWTH FACTOR BINDING TO HUMAN COLORECTAL CANCER CELLS (MEETING ABSTRACT).

StHilaire RJ, Kim YS
Gastrointestinal Research Lab., Veterans Administration Medical Center, San Francisco, CA, 94121
Gastroenterology; 80(5,part2):1267 1981

Epidermal Growth Factor (EGF), a 6000 mol wt protein hormone produced by salivary and Brunner's glands, has been shown to have a mitogenic effect in normal intestine. Work in our laboratory has demonstrated that EGF stimulates the growth of human adenocarcinoma cells in culture as well as binding to a 180,000 mol wt cell surface receptor. Sodium butyrate (NaBt) has been reported to induce differentiation in some cancer cell systems. In this study a human rectal carcinoma cell line (HRT-18) and a human colon adenocarcinoma cell line (HT-29), whose growth is stimulated by EGF, were grown in the presence of NaBt to assess alterations in growth, tumorigenicity, EGF receptor numbers and characteristics. NaBt treated cells in both cell lines exhibited longer doubling times, lower saturation densities, and an inability to demonstrate anchorage independent growth when compared with control nontreated cells. A significant increase in receptor numbers was noted when expressed as binding per well or binding per mg protein. HRT-18 demonstrated a two-fold increase while HT-29 demonstrated a 1.3 fold increase after incubation with 2mM NaBt. This increase appears to require protein synthesis. Gel filtration and lectin affinity chromatography suggested that the EGF-receptor complex is a glycoprotein. In summary, NaBt can cause changes in cell growth in human colon cancer cells, suggesting lower tumorigenicity, while at the same time causing quantitative changes in EGF receptors. (no Refs)

3. DIFFERENTIAL MODULATION OF HUMAN CHORIONIC GONADOTROPIN SECRETION BY EPIDERMAL GROWTH FACTOR IN NORMAL AND MALIGNANT PLACENTAL CULTURES.

Huot RI, Foidart JM, Nardone RM, Stromberg K
(c/o Stromberg) Lab. Tumor Viral Carcinogenesis, NCI, Frederick Cancer Res. Center, Building 560, Room 12-70, Frederick, MD, 21701
J Clin Endocrinol Metab; 53(5):1059-1063 1981

The ability of epidermal growth factor (EGF) to modulate the secretion of human chorionic gonadotropin (hCG) in both normal and malignant placental cells was compared. Receptors for EGF wer e present on the JAr line of choriocarcinoma cells and were localized to the trophoblast cells of normal placental organ cultures as detected by immunofluorescence. Despite the presence of EGF receptors, the normal placenta did not respond to EGF by significantly increasing its levels of hCG production. The JAr line of choriocarcinoma exhibited a two-fold increase in hCG secretion after the addition of EGF. EGF stimulated growth in the JAr cells, as measured by the protein content of the cultures, but did not elevate the incorporation of [methyl-3H]thymidine in either the JAr cells or placental organ cultures. (Author abstract) (25 Refs)

4. MECHANISM OF SYNERGISTIC INDUCTION OF DNA SYNTHESIS BY EPIDERMAL GROWTH FACTOR AND TUMOR PROMOTERS.

Matrisian LM, Bowden GT, Magun BE
Dept. Anatomy, Univ. Arizona Health Sciences Center, Tuscon, AZ, 85724
J Cell Physiol; 108(3):417-425 1981

The effects of a number of phorbol ester compounds with varying tumor-promoting abilities on epidermal growth factor (EGF) binding, degradation, and stimulation of DNA synthesis was studied in the Rat-1 cell line (F2408 established line derived from Fischer rat embryo fibroblasts). The phorbol esters alone were able to induce a small increase in the amount of DNA synthesis compared to Me2SO-treated control cells. In combination with high concentrations of EGF (1-10 nanog/ml), the effect of phorbol esters on EGF-induced DNA synthesis was less than additive. However, at EGF concentrations between 0.001 and 0.05 nanog/ml, there was a marked synergism between EGF and phorbol esters. 12-O-tetradecanoyl phorbol-13-acetate (TPA) exhibited the greatest degree of synergism at low EGF concentrations. The observed increase in DNA synthesis in the presence of TPA and EGF was due to a TPA-induced increase in the overall rate of synthesis. The inhibition of EGF binding to Rat-1 cells by TPA and other phorbol ester tumor promoters was dose dependent. The ability of the various phorbol esters to spare EGF from degradation and to act synergistically with EGF to induce DNA synthesis correlated with the tumor-promoting ability of the compounds. In addition, the concentration of EGF at which there was a significant increase in the amount of EGF spared from degradation in phorbol ester-treated cells, compared to controls, correlated with the EGF concentration at which EGF and phorbol esters exerted synergistic effects on DNA synthesis. (31 Refs)

5. ENHANCEMENT OF CALCIUM UPTAKE AND PHOSPHATIDYLINOSITOL TURNOVER BY EPIDERMAL GROWTH FACTOR IN A-431 CELLS.

Sawyer ST, Cohen S
Dept. Biochemistry, Vanderbilt Univ. Sch. Medicine, Nashville, TN, 37232
Biochemistry; 20(21):6280-6286 1981

Epidermal growth factor (EGF) stimulates the incorporation of 32Pi and [3H]inositol into phosphatidylinositol (5-10-fold) in A-431 cells. EGF also stimulates the incorporation of 32Pi into p hosphatidic acid (up to 10-fold). These effects are attributed to an acceleration of the turnover of phosphatidylinositol as a consequence of the binding of EGF to its membrane receptor. The extent of the phosphatidylinositol response to EGF parallels the extent of hormone binding. The phosphatidylinositol response to EGF appears to be dependent on an influx of calcium since (1) external calcium is required for the enhancement of phosphatidylinositol turnover, (2) the accumulation of 45Ca by A-431 cells is stimulated by EGF, (3) blockage of calcium influx with LaCl3 inhibits stimulation of phosphatidylinositol turnover, and (4) calcium influx via ionophore A23187 is sufficient to stimulate phosphatidylinositol turnover. Since the binding, internalization, and degradation of 125I-labeled EGF

in A-431 cells are unaffected by the omission of calcium from the medium, external calcium and phosphatidylinositol turnover are not necessary for the internalization and degradation of the EGF-receptor complex. (Author abstract) (31 Refs)

6. CELL CYCLE ARREST POINTS IN NONTUMORIGENIC AND TUMORIGENIC CELL LINES, DEPENDENCY ON GROWTH FACTORS (MEETING ABSTRACT).

Meiss HK, Marcus M
Department of Cell Biology, New York University Medical School, New York, NY
Growth of Cells in Hormonally Defined Media, September 1-4, 1981, Cold Spring Harbor, New York Cold Spring Harbor Laboratory 101 pp., 1981.

We found that the ability of the TM4 cells to traverse G1 depends upon epidermal growth factor (EGF) more than upon insulin (ins) or transferrin (trf) as illustrated by the fact that in cultures starved for 3 days for EGF, 86% of the cells are arrested specifically in late G1, while omission of ins or trf for 3 days resulted in the arrest in G1 of only 61 and 75% of the cells, respectively. When the cells are starved for isoleucine or serum, 61% of TM4 cells are arrested in late G1. However, in presence of ins and trf, 0.3% serum supports growth. Therefore, 0.3% serum seems to provide what EGF provides. The tumorigenic derivative of TM4, designated TM4-T which cannot grow in serum free (sf) medium was also analyzed for its growth requirements. We found that more than 90% of these cells are arrested in G1 when starved for either serum or isoleucine. However, whereas TM4 cells under similar starvation conditions are arrested in late G1, TM4 cells are arrested in early G1. Hittelman and Rao have shown that in confluent cultures contact inhibited cells are arrested in early G1 while transformed cells under similar conditions are arrested in late G1. We found that the tumorigenic rat Sertoli cell line TRST is less dependent upon the growth factors than is the nontumorigenic line TM4. In cultures starved for 3 days for either EGF or trf only 61% of the cells are arrested in late G1, while in cultures starved for 3 days for ins the distributi on of cells is similar to that in the random cultures. However, in TRST cultures starved for serum or isoleucine the fraction of arrested cells in late G1 is similar to starved TM4 cells. (1 Ref)

7. HIGH MOLECULAR WEIGHT GROWTH FACTOR(S) IN THE CONDITIONED MEDIUM OF 7,12-DMBA RAT MAMMARY ADENOCARCINOMA: SPECIFIC BINDING TO EGF RECEPTORS (MEETING ABSTRACT).

Salomon D, Kidwell WR, Kohn E, Zwiebel JA
Laboratory of Pathophysiology, National Cancer Institute, National Institutes of Health, Bethesda, MD, 20205
Thirteenth Miami Winter Symposium: Cellular Responses to Molecular Modulators, January 12-16, 1981, Miami, Florida. University of Miami 112 pp., 1981.

Murine sarcoma virus(MSV)-transformed cells have been demonstrated to possess a reduced number of epidermal growth factor (EGF) receptors when compared to their nontransformed counterparts. This led to the discovery of sarcoma growth factor (SGF) in the conditioned medium of MSV-transformed cells. Using a similar strategy, we have found that 7,12-Dimethyl-benzanthracene (DMBA) rat mammary adenocarcinoma (RMA) cells, which are grown in primary culture in hormone defined, serum free medium, possess fewer EGF receptor sites when compared with normal rat mammary epithelial (RME) cells (25,000 versus 84,000 sites per cell, respectively). The conditioned medium (RMA-Cm) from these primary mammary cultures contains a factor(s) which competes with [125I]-EGF, but not with [125I]-insulin, for binding to its receptor on OTT-6050 mouse embryonal carcinoma cells. When the RMA-CM is filtered in 1% acetic acid, the EGF competition activity resides in 100-300 K and greater than 300K mol wt Diaflo ultrafiltration fractions. Like SGF, the RMA-CM factor(s) is acid soluble and protease sensitive. Unlike SGF, the RMA-CM factor is heat labile. In terms of its biological effects, RMA-CM is similar to SGF. The factor(s) in RMA-CM stimulates the monolayer growth of rat NRK-49F (normal rat kidney) and normal RME cells. It reduces the serum requirement of RME cells and promotes the anchorage-independent growth of NRK-49F cells in soft agar. These studies demonstrate the presence of a high mol wt factor(s) in the conditioned medium of chemically induced rat mammary adenocarcinoma cells. The factor competes specificially for EGF receptors and, like SGF, can transform normal cells - at least with respect to their ability to grow in soft agar. Both mesenchymal and epithelial cell types respond to the growth-promoting activity in RMA-CM, which may function to promote tumor cell growth as well. (1 Ref)

8. SPECIFIC BINDING OF TRANSFORMING GROWTH FACTOR CORRELATES WITH PROMOTION OF ANCHORAGE INDEPENDENCE IN EGF RECEPTORLESS MOUSE JB6 CELLS.

Colburn NH, Gindhart TD
Lab. Viral Carcinogenesis, NCI, Frederick, MD, 21701
Biochem Biophys Res Commun; 102(2):799-807 1981

Promotion of anchorage independence by a transforming growth factor is reported. JB6 mouse epidermal cells were exposed in vitro to 12-O-tetradecanoyl-phorbol-13-acetate (TPA: 1.6 x 10(-8) M), epidermal growth factor (EGF: 2 or 20 nanog/ml), or transforming growth factor (TGF: 5 ug/ml) derived from a human rhabdomyosarcoma line. Binding was measured using 125I-labeled EGF and TGF. Some clones of JB6 cells, such as clone 41, were sensitive to both EGF and TPA, while others were resistant to both. Sensitivity was measured by colony formation in 0.33% agar. Most of the colonies produced by TPA, EGF, or TGF gave rise to transformed cell lines which formed colonies in agar without inducer. A TPA-resistant derivative cell line of clone 41, R219, did not bind EGF specifically. Anchorage independence could be induced in R219 by TPA or TGF, but not by EGF. These cells appeared to have a class of binding sites specific for TGF in addition to sites binding EGF. The EGF sites, which also bind TGF, evidently were not necessary for transformation by TGF. (16 Refs)

9. NEW CLASS OF TRANSFORMING GROWTH FACTORS POTENTIATED BY EPIDERMAL GROWTH FACTOR: ISOLATION FROM NON-NEOPLASTIC TISSUES.

Roberts AB, Anzano MA, Lamb LC, Smith JM, Sporn MB
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Proc Natl Acad Sci USA; 78(9,part2):5339-5343 1981

Proteins potentiated by epidermal growth factor (EGF) to induce a transformed phenotype in non-neoplastic rat kidney fibroblasts in cell culture have been isolated from many non-neoplastic tissues of the adult mouse, including submaxillary gland, kidney, liver, muscle, heart, and brain. They resemble previously described transforming growth factors (TGFs) isolated from neoplastic cells as follows: they are extractable by acid/ethanol and are acid-stable, low mol wt (6,000-10,000) polypeptides requiring disulfide bonds for activity, and they cause anchorage-independent growth of non-neoplastic indicator cells

that will not grow in soft agar in their absence. Partial purification of these TGFs from submaxillary glands of male mice shows that they are distinct from EGF. Unlike previously described extracellular TGFs, but like certain cellular TGFs from neoplastic cells, they are potentiated by EGF in their ability to promote anchorage-independent growth. The isoelectric point of the submaxillary gland TGF protein is near neutrality. Chromatography on Bio-Gel P-30 followed by high-pressure liquid chromatography has resulted in a 22,000-fold overall purification. The most purified protein is active in inducing growth in soft agar in 1 ng/ml when assayed in the presence of EGF. The data add further evidence to the concept that neoplasia may result from a quantitative, rather than qualitative, alteration in non-neoplastic biochemical processes. (Author abstract) (25 Refs)

10. IN VITRO SYNTHESIS OF NERVE GROWTH FACTOR SUBUNITS BY MOUSE SARCOMA LINE S180 (MEETING ABSTRACT).

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Thirteenth Miami Winter Symposium: Cellular Responses to Molecular Modulators, January 12-16, 1981, Miami, Florida. University of Miami 112 pp., 1981.

The nerve growth factor (NGF) is necessary for the differentiation and maintenance of vertebrate sympathetic and embryonic sensory neurons. When isolated from mouse submaxillary gland, human placenta at term or guinea pig prostrate, NGF is a subunit containing protein. In mouse submaxillary gland, NGF is made up of three dissimilar subunits called alpha, beta and gamma. The beta subunit contains all of the nerve growth promoting activity. The gamma subunit expresses arginyl-esteropeptidase activity and has been suggested to process larger pro-beta forms into the active beta species. In order to better define the processing properties of gamma NGF and study regulatory features of the synthesis and processing of NGF, we studied the synthesis of NGF by a mouse S180 sarcoma cell line in vitro. NGF subunits synthesized de novo in the presence of 35S-methionine were isolated by monospecific antibodies bound to sepharose. We were able to show that the S180 line synthesized alpha, beta and gamma molecules indistinguishable from alpha, beta and gamma NGF isolated from mouse submaxillary gland in terms of antigenic properties, size and charges properties as well as biological activities. This is the first demonstration that the subunits of NGF are synthesized and secreted as a 7S complex similar to that isolated in the mouse submaxillary gland. (no Refs)

11. NERVE GROWTH FACTOR: ITS DISTRIBUTION, CHEMISTRY, AND FUNCTION.

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Shengli Kexue Jinzhan; 12(2):132-138 1981

The distribution, biochemistry, biological functions, and mechanisms of action of nerve growth factors (NGFs) are reviewed. This isolation from the murine submaxillary gland and from snake venoms and the determination of their structures are described in detail. The male submaxillary gland contains by far the greatest amount of NGF, greater than that in the female submaxillary gland and in other organs. (20 Refs) (In CHI)

12. FACTORS FROM TISSUE CULTURE OF ASTROCYTOMAS STIMULATE PROLIFERATION OF CULTURED VASCULAR ENDOTHELIAL CELLS (MEETING ABSTRACT).

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Acta Neurochir; 56(1/2):126 1981

Endothelial cell proliferation and neovascularization of astrocytomas are well-known characteristics of these neoplasms. The formation of tumor blood vessels seems to be dependent on the mig ration and proliferation of vascular endothelial cells. Both of these functions appear to be controlled by specific chemical factors produced by tumor cells and tumor angiogenesis factor (TAF). We have cultured biopsy astrocytoma cells of various degrees of malignancy in tissue culture for periods of up to several wk. Supernatants from these cultures were harvested at various intervals, and their ability to enhance proliferation of cultured umbilical vein human endothelial cells was evaluated. All of the supernatants tested contained some degree of TAF activity, and an increase in endothelial cell proliferation from 1.5 to 3 x control cells was found. No correlation between the degree of TAF activity and the histological malignancy criteria of the tumor cells could be found. The supernatants seemed to contain max TAF activity after approx 2-5 days in culture and gradually decreased thereafter. Supernatants from fibroblast monolayer controls did not seem to be significantly stimulatory for the endothelial cells model used here. On the other hand, supernatants from the astrocytoma cultures did increase the growth of human skin fibroblast in culture but to a lesser degree than that found for endothelial cells. We conclude that cultured astrocytoma cells produce a mitogenic factor which specifically enhances growth of human endothelial cells in vitro. (no Refs)

13. RELEASE OF AN AUTOSTIMULATORY GROWTH ACTIVITY BY HUMAN MALIGNANT MELANOMA CELLS (MEETING ABSTRACT).

Richmond A, Lawson DH, Nixon DW
Department of Medicine, Emory University School of Medicine, Atlanta, GA
Growth of Cells in Hormonally Defined Media, September 1-4, 1981, Cold Spring Harbor, New York Cold Spring Harbor Laboratory 101 pp., 1981.

We examined concentrates of Hs0294 conditioned medium (CM) and acid-ethanol extracts of this conditioned medium (CMae) for ability to stimulate (3H)-thymidine incorporation and increase cell number in other Hs0294 cultures. When 8-32 ug of CM protein was tested for ability to stimulate (3H)-thymidine incorporation, the minimal effective dose (MED) was 16 ug and higher concentrations of this preparation did not increase the stimulatory effect. Daily addition of 25 ug of CM protein to serum-free cultures increased cell number 40% above that of control cultures after four days of treatment. This same concentration of CM elevated (3H)-thymidine incorporation 71% above that of control. The MED for this preparation was 5.5 ug, with 11 ug producing a half-maximal response and 22 ug producing the maximal response. Twenty-four ug of CMae stimulated the incorporation of (3H)-thymidine into DNA to 100% greater than control and increased cell number to 55% greater than control. Although CMae from early passage primary cultures of a non-malignant nevus did not contain melanoma growth stimulatory activity (MGSA), these cells did respond to this substance. The activity is stable to IN acetic acid at 25C for 48 hr and stable to boiling for 10 min. Acid-ethanol extracts of 5 human melanoma tumors

also contained MGSA, as determined by ability to stimulate (3H)-thymidine incorporation in Hs0294 cells. The MED of these tumor extracts ranged from 1-10 ng, producing maximal stimulation of (3H)-thymidine incorporation 51-130% greater than control. Thus, MGSA may contribute to the proliferation of tumor cells in vivo and in vitro. (no Refs)

14. GENETIC AND ONCOGENIC INFLUENCES ON MYELOPOIESIS.

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Haematol Blood Transfus; 26:237-242 1981

Murine myelomonocytic leukemic cell line WEHI-3 obtained from Balb/c mouse was studied. Tumor was composed of monocytic and granulocytic cells, which on transplantation developed four distinct sublines. The most neoplastic feature of the WEHI-3 myelomonocytic cells was that most of the factors were produced constitutively rather than as a result of lymphokine or adjuvant induction. Another feature of leukemic cells was production of G-, GM-, M-, and colony stimulating factor (CSF), which stimulate leukemic cells to proliferation and differentiation. The combination of growth factor production and simultaneous presence of growth factor receptors on the surface of the same cells is rare. ***Tumor cell autostimulation*** by its own growth factors could be more widespread. This continuous endogenous production of growth factors induces continuous and unchecked proliferation. WEHI-3-conditioned medium (WEHI-3-CM) activities produced CSF, some striking differences in marrow colony-forming units-culture were observed. Possibility of defects in CSF molecule, growth factor receptors or all the way back in pluripotential stem cell were presented and discussed. (26 Refs)

15. SENSITIVITY OF ACUTE MYELOID LEUKAEMIA CELLS TO COLONY STIMULATING ACTIVITY: RELATION TO RESPONSE TO CHEMOTHERAPY.

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Br J Haematol; 49(2):259-267 1981

Agar cultures of acute myeloid leukemia (AML) cells were used to determine the sensitivity of AML clone forming cells to the specific regulator granulocyte-macrophage colony stimulating activity (CSA). The mean CSA threshold (amount of CSA required to stimulate 0.5 of the clone forming cells) of 27 different AML cell samples varied from normal to an approx nine-fold increase in CSA requirement. The degree of sensitivity to CSA at presentation was found to be closely correlated to the response to induction chemotherapy in 15 patients. There was a highly significant correlation (p less than 0.001) between the mean CSA threshold and the number of courses of chemotherapy required to induce remission. Also, an inc reased mean CSA threshold at presentation was associated with a significantly slower decline of marrow blasts following the first course of chemotherapy and a reduced remission rate. Sensitivity to CSA was closely related to the clone size achieved in maximally stimulated cultures of AML cells and this in turn also related to the remission rate and ease with which remission was achieved (presentation marrows, 39 patients). Possible reasons for the linking of these two functional properties of AML cells and their relation to response to chemotherapy will be discussed. (Author abstract) (12 Refs)

16. GRANULOPOIESIS ASSOCIATED WITH THE C3HBA TUMOR IN MICE.

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JNCI; 67(5):1135-1138 1981

Female C3H/HeJ mice were inoculated with syngeneic breast adenocarcinoma cells (C3HBA). A progressive neutrophilia developed as the tumors grew. A linear relationship was demonstrated between the tumor diameter and the extent of the neutrophilia. Local tumor excision caused a rapid fall (3 days) in the neutrophil count. Media conditioned with tumor cells, normal mouse kidney, and bone marrow of normal or tumor-bearing mice were prepared. Tumor cell-conditioned medium was found to have marked stimulating activity for granulocytic colony formation of mouse bone marrow cells. Sera from tumor-bearing mice also had colony-stimulating activity. This finding strongly suggested that the neutrophilia was caused by the release of a ***neutrophil-stimulating factor*** from the tumor. (Author abstract) (11 Refs)

17. REGULATION OF HUMAN T-CELL PROLIFERATION: T-CELL GROWTH FACTOR AND ISOLATION OF A NEW CLASS OF TYPE-C RETROVIRUSES FROM HUMAN T-CELLS.

Gallo RC, Poiesz BJ, Ruscetti FW
NCI, Bethesda, MD, 20014
Haematol Blood Transfus; 26:502-514 1981

Recent results with human T-cell growth factor (TCGF), the various T cell systems, and the isolation of a new type-C retrovirus released from growing T-cells from some of these T-cell neoplasias are summarized. Unlike normal T-cells, neoplastic mature T-cells respond directly to TCGF without prior lectin or antigen in vitro activation. Transformed T-cell lines exhibit phenotypic characteristics consistent with the neoplastic cells of their disease of origin. A new retrovirus, human T-cell lymphoma-leukemia virus (HTLV) was isolated from fresh and cultured cells from two patients, each with a variant of a cutaneous T-cell lymphoma. Further studies showed that HTLV was not significantly related to any known animal retrovirus, is not an endogenous (genetically transmitted) virus of man, and at present is only associated with fresh or cultured T-cells from patients with the T-cell neoplasia. The results suggest that HTLV infected some mature T-cells of some people and may be involved in some neoplasias involving these cells. (33 Refs)

18. THE SPECIES SPECIFICITY OF INTERLEUKIN-2 (MEETING ABSTRACT).

Lindsay P, Schwulera U, Sonneborn HH
BIOTEST - Serum - Institut Gmbh, Geleitsstrasse 103, D-6050 Offenbach, W. Germany
Third International Symposium on Human Lymphokines, October 15-17, 1981, Dallas Texas Wadley Institutes of Molecular Medicine; 1981

Conditioned media obtained by the polyclonal activation of lymphocyte cultures, or, more recently, T cell-derived tumor lines have been found to contain a factor which allows for long-term growth and maintenance of antigen-activated or committed T cells in the absence of continued antigenic stimulation. This T cell growth factor, now referred to as Interleukin-2 (IL-2), has been extensively studied by numerous laboratories and is biochemically defined for several species. Our present study has investigated the functional activity of Interleukin-2 across MHC-barriers. IL-2 containing supernatants de-

rived from man, goat, guinea pig or rabbit, function effectively on mouse test cells, with the human-derived supernates exhibiting especially high activity. In contrast, Interleukin-2 derived from murine lymphocytes or cell lines is ineffective in either promoting or maintaining human T cell growth. This makes human IL-2 containing supernates the reagent of choice when considering various experimental systems as it does not exhibit the species restriction exhibited by the murine class of T cell growth factors. (no Refs)

19. SPONTANEOUS PRODUCTION OF T CELL GROWTH FACTOR BY A CONTINUOUS LINE OF GIBBON TUMOR T CELLS (MEETING ABSTRACT).

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In Vitro; 17(3):213 1981

T cell growth factor (TCGF) or Interleukin 2 (IL2) is a lymphokine which supports the in vitro replication of activated T cells. Certain murine and human T cell lines can be activated to produce TCGF by treatment with lectins, phorbol-12-myristrate-13-acetate (PMA) or a combination of lectin and PMA. Some human T cell lines after becoming independent of TCGF have been found to produce levels of TCGF without activation. A continuous T cell line, MLA144, originally established from a gibbon with lymphosarcoma, was found to release spontaneously high levels of TCGF. Conditioned media (CM) from MLA144 cells supports the growth and DNA synthesis of T cells from man, several other species of primates, and from mice. The factor in MLA144 CM has a mol wt, as determined by gel filtration, of 21,500 to 24,000 daltons, elutes from diethylaminoethyl-cellulose at 0.04 to 0.06 M sodium phosphate buffer, pH 7.6, is resistant to 60 C and to low and high pH. MLA144 cells form rosettes with sheep RBC and lack the receptor for the Fc portion of IgG. (no Refs)

20. A MITOGENIC FACTOR FOR TRANSFORMED CELLS FROM HUMAN PLATELETS (MEETING ABSTRACT).

Lipton A, Kepner N, Witkoski E, Rogers C, Leitzel K
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Chesapeake Conference on Hemostasis and Malignancy-Interaction of Platelets and Tumor Cells, June 26-30, 1981, St Mary's Maryland American Red Cross; 1981

Extracts of human platelets stimulate the in vitro multiplication of both 3T3 mouse fibroblasts and SV40-transformed mouse fibroblasts (SV3T3). The first evidence that there might be several factors present in platelet extracts (platelet-derived growth factor, PDGF) came from preliminary attempts to characterize the platelet mitogen. Growth-promoting activity in crude platelet extracts for 3T3 cells (PDGF1) was stable on heating to 100 degrees for 5 min. In contrast, growth-promoting activity for SV3T3 cells (PDGF2) was destroyed by heating at 100 degrees for 5 min. PDGF2 is destroyed by treatment with sodium periodate (0.05 mM at 4 degrees for 48 hr), is partially destroyed by trypsin treatment, and adsorbs to Concanavalin A Sepharose. It would thus appear to be a glycoprotein. PDGF2 is non-dialyzable and on Sephadex G-100 gel column at pH 7.4 has a mol wt of 72,000 daltons. Its isoelectric point is between 7.8-8.3. In this regard, PDGF2 also differs from PDGF1, which focuses between pH 9.6-9.8. Platelet extracts which contain PDGF2 activity can promote in vivo growth of Herpes Simplex Virus 2-(HSV-2) transformed hamster embryo fibroblast cells in weanling Syrian hamster. In summary, human platelets contain a heat-labile, non-dialyzable glycoprotein with an isoelectric point between 7.8-8.3 that can promote the in vitro and in vivo growth of a variety of transformed cells. (no Refs)

21. EFFECT OF PLATELET LYSATE ON GROWTH OF HUMAN TUMOR COLONIES (MEETING ABSTRACT).

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Chesapeake Conference on Hemostasis and Malignancy-Interaction of Platelets and Tumor Cells, June 26-30, 1981, St Mary's Maryland American Red Cross; 1981

To determine whether platelets might affect metastasis formation of human cancers by altering tumor cell growth, we cultured cells from solid tumors in the presence and absence of platelet factors (PF) obtained by freeze-thaw lysis or collagen or thrombin stimulation using the human tumor stem cell assay (HTSCA). PF obtained by lysis increased uptake of 3H-thymidine (3HTdR) by 3T3 cells grown in monolayer culture by 300%; released PF increased 3HTdR uptake by 3T3 cells 100%. PF obtained by lysis or release increased 3HTdR uptake by SV40-transformed 3T3 cells 150%. Increases were dose dependent. PF was non-di alysable, labile to 70 C for 1/2 hr, and partially trypsin sensitive. One hundred forty-seven of 208 malignant tumors (71%) grew 5 or more colonies in the 2-layer soft agar culture system. The viability of the cells cultured range from 10-90% and the plating efficiency ranged from 0.001-0.35%. Addition of PF (200 ug protein/ml) to the underlayer of the culture system increased tumor colony forming units (TCFU) from 84 tumors by 60--350% and decreased TCFU from 45 tumors by 13-65%. PF had no effect on TCFU from 12 tumors. The peak response to PF occurred after 3-10 days in culture. Cell viability and the number of cells plated did not affect plating efficiency or the response to PF. The results indicate that the HTSCA can be used to assess the response in vitro of TCFU to PF. Platelet factors may both stimulate and inhibit the growth of TCFU; presumably different factors mediate these different responses. These factors may affect the growth of tumor cells lodged at metastatic sites and may therefore influence metastasis formation. (no Refs)

22. INTERACTION OF PLATELETS AND TUMOR CELLS (MEETING ABSTRACT).

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Chesapeake Conference on Hemostasis and Malignancy-Interaction of Platelets and Tumor Cells, June 26-30, 1981, St Mary's Maryland American Red Cross; 1981

In a series of studies, certain tumor cells were shown capable of aggregating platelets, while platelets undergoing aggregation were found to support tumor cell proliferation. Two lines of mouse tumor cells (renal adenocarcinoma, RAG and neuroblastoma, Neuro-2a) capable of aggregating mouse or rabbit platelets were investigated. Specific divalent cation requirements were identified for the tumor cell-induced platelet aggregation. This activity was associated with the tumor cell membrane and was also characterized by very specific structural constraints. Aggregation was accompanied by a secretory release reaction, products of which were found to have growth-promoting activity for tumor cells. Lysates of human platelets provided an excellent source for this factor, which was able to support tumor cell proliferation in the absence of plasma or serum. The activity was non-dialysable, heat-stable at 56 degrees, partially trypsin-labile and sensitive to periodate oxidation. One or more

peptides with strong growth-promoting activity coud be isolated by isoelectric focusing. These peptides are not identical with the platelet-derived growth factor with activity for non-malignant cells. (no Refs)

23. TRANSFORMING GROWTH FACTORS (TGFS) PRODUCED BY TUMOR CELLS (MEETING ABSTRACT).

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In Vitro; 17(3):213 1981

Cells tranformed by RNA-containing tumor viruses, as well as certain human tumor cells in culture (sarcomas, carcinomas and melanomas), release potent growth stimulating peptides into the culture medium. These proteins are acid and heat stable, produce profound morphologic changes in rat and human fibroblasts and enable normally anchorage-dependent cells to grow in semi-solid medium. Removal of the factors results in a reversion of cell phenotype. The larger TGF has an apparent mol wt of 20,000-23,000 daltons, while the smaller forms range from 7,000-10,000 daltons. The initial effect on cells of each of the forms involves an interaction with epidermal growth factor (EGF) cell membrane receptors. They compete with 125I-EGF for binding sites on the receptor. The EGF receptor is phosphorylated on tyrosine residues as a result of TGF binding; certain lower mol wt proteins also are phosphorylated following TGF binding. TGFs from both rodent and human cells have been purified by gel filtration, ion exchange chromatography and high pressure liquid chromatography; they are active in producing colony formation and the transformed phenotype at less than 1 ng/ml. Since tumor cells produce TGFs and have receptors that enable them to respond, it is suggested that these peptides play a role in facilitating the growth and progression of tumors under natural conditions. (no Refs)

24. PRODUCTION OF TUMOR ANGIOGENESIS FACTOR (TAF) BY HUMAN TUMOR CELL LINES (MEETING ABSTRACT).

Tolbert WR, Kuo MJ, Feder J
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In Vitro; 17(3):259 1981

Folkman and others have reported that tumor cells release an angiogenic material which stimulates the growth of host vessels and their perfusion of the tumor mass. This tumor angiogenesis factor (TAF) has been isolated from fresh minced or primary cultured animal and human tumors and assayed on the chorioallantoic membrane (CAM) of the chick embryo. A few long-term rodent cell lines and SV40 transformed human lines also have shown positive TAF activity. In order to provide sufficient material for isolation and purification of a human TAF, seven cell lines derived from a wide variety of spontaneous human tumors were selected both for CAM activity and efficient large-scale suspension growth potential. The lines A375 melanoma, HT-29 colon adenocarcinoma, MG-63 osteosarcoma, Raji Burkitt lymphoma, RD embryonal rhabdomyosarcoma, SK-HEP-1 liver adenocarcinoma and TE-671 medulloblastoma were all grown to greater than 200 liters and CAM-active TAF was isolated. (no Refs)

25. PURIFICATION OF AN ANGIOGENIC/ ENDOTHELIAL CELL GROWTH STIMULATORY FACTOR FROM THE WALKER 256 RAT TUMOR (MEETING ABSTRACT).

Watt S, Wallis K, Fenselau A
Department of Physiological Chemistry, The Johns Hopkins University School of Medicine, Baltimore, MD
Thirteenth Miami Winter Symposium: Cellular Responses to Molecular Modulators, J anuary 12-16, 1981, Miami, Florida. University of Miami 112 pp., 1981.

Previously we reported the stimulation of growth of fetal bovine aortic endothelial cells by crude homogenates of Walker 256 rat tumor. We now report the details of the purification of the factor responsible for this activity. Homogenates of Walker 256 rat tumor cells, prepared by sonication of a suspension of ascitic tumors cells, were used as starting material. Activity was determined using our in vitro assay system, where growth stimulatory effects on fetal bovine aortic endothelial cells were monitored by measuring actual cell counts or incorporated 3H-thymidine. In vivo angiogenic activity was determined using the chicken chorioallantoic membrane (CAM) or rat corneal implant assay. Acid (pH4.9) treatment and elution from a Bio-Gel P-30 column at pH 3.9 yielded only one active component with an apparent mol wt of approx 300-400 daltons. Thereafter, purification of the stimulatory material was achieved by extracting lyophilized crude tumor homogenates with ethanol. This ethanol extract, which retained all the initial crude activity, was then further purified by silica gel chromatography. Active material eluted with ethyl acetate:methanol (3:1) and appeared to consist of 3-4 components by reversed-phase high pressure liquid chromatography (HPLC) and by thin layer chromatography (TLC) on PEI-cellulose or silica gel. Purification to homogeneity (by HPLC and TLC) was accomplished by rechromatography on silica gel. The purified material was found to stimulate vessel growth in a dose-dependent manner when tested on the CAM and rat corneal implant bioassays. Thus, it is both an endothelial cell growth factor and an angiogenic factor. (1 Ref)

26. ANGIOGENESIS IN MALIGNANT TUMORS.

Novak F
Salmovska 3, 120 00 Praha 2, Czechoslovakia
Cas Lek Cesk; 120(33):983-986 1981

A tumor in the initial stages has no blood supply of its own and obtains nutrients by diffusion. A diffusable tumor angiogenic factor (TAF), which has been isolated from experimental and human cancers, causes vascularization of tumors. Antibodies to TAF and methods of TAF detection have been developed. (no Refs) (In CZE)

27. MITOGENIC FACTORS PRESENT IN SERUM BUT NOT IN PLASMA.

Balk SD, Levine SP, Young LL, LaFleur MM, Raymond NM
Dept. Pathology, Univ. Manitoba, Winnipeg, R3E OW3, Canada
Proc Natl Acad Sci USA; 78(9,part2):5656-5660 1981

In culture medium containing heparinized, heat-inactivated, chicken plasma, normal chicken heart mesenchymal cells do not proliferate but their Rous sarcoma virus-infected counterparts proliferate maximally. In medium containing serum derived from chicken whole blood or plasma, on the other hand, normal chicken heart mesenchymal cells proliferate actively, at similar overall rates and to similar extents. The rate and extent of normal cell proliferation are decreased by a factor of approx 1/2 with whole blood-derived serum that is heparinized and in-

activated; proliferation ceases in plasma-derived serum that is heparinized and inactivated. Heparinization and inactivation of serum does not affect the proliferation of Rous sarcoma virus-infected cells, indicating that this combined treatment eliminates a mitogenic (regulatory) rather than a supportive (nutrient) factor(s) for cell replication. We hypothesize that mitogen(s) is released from plasma protein precursors when plasma clots in the presence of formed elements of the blood or when plasma-derived serum is exposed to cultured cells; heparinization and inactivation, within the framework of this hypothesis, would render nonfunctional the plasma protein precursor(s) from which the mitogen(s) is generated. Alternatively, our data are consistent with the release of two mitogens during blood clotting, one from plasma protein precursors and the other from formed elements of the blood. We also have studied the proliferative behavior of Swiss and BALB/c3T3 cells in whole blood-derived and plasma-derived human serum. Our studies suggest that the platelet-derived growth factor has an artifactual supportive (nutrient) role, rather than an authentic mitogenic role, in cell replication. (Author abstract) (31 Refs)

28. STUDIES IN VITRO ON THE INDUCTIVE ACTION OF TUMOUR TISSUES ON THE ECTODERM OF CHICK BLASTODERM.

Zhang J
Dept. Cell Biology, Cancer Inst., Chinese Acad. Medical Sciences, Beijing, People's Republic of China
Acta Anat Sin; 12(2):192-196 1981

The ability of tumor tissues in inducing cell proliferation and cell differentiation in the ectoderm of chick blastoderm is described. Both fresh tumor tissues and those treated with 70% alcohol were used. Tumors used included Rous sarcoma, rat Walker mammary carcinoma, murine spindle cell sarcoma, murine sarcoma 180, and human esophageal carcinoma (ECA-109). Results showed that only fresh tissues from sarcoma 180 and alcohol-treated tissues from spindle cell sarcoma were able in inducing neural formation in the ectoderm of chick blastoderm. (19 Refs) (In CHI)

29. THE EFFECT OF ADDED H1 HISTONE AND POLYLYSINE ON DNA SYNTHESIS AND CELL DIVISION OF CULTURED MAMMALIAN CELLS.

Kundahl E, Richman R, Flickinger RA
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J Cell Physiol; 108(3):291-298 1981

The effect of adding whole histone, various histone fractions, polylysine, and polyarginine on the rate of cell division and DNA synthesis of three murine cell lines (cultured Friend erythroleukemia cells and two mouse lymphoma cell lines, el-4 and S-49) was studied. Low levels (10 ug/ml) of H1 histone or polylysine stimulated DNA synthesis by the creation of additional initiation sites for repl ication and production of smaller av replicon sizes. Untreated Friend erythroleukemia cells underwent a decrease in cell division rate on days 2-5 of culture while the decrease in rate of cell division was much less for cells treated with H1 histone or polylysine. This might mean that the observed results are not due to the stimulation of cell division but rather to a continuation of cell division in the H1 and polylysine-treated cultures. However, the data from the experiments with the lymphoma cells showed clearly that H1 histones can stimulate the rate of cell division before the rate of cell division of the untreated cells began to plateau. In the lymphomas, there was a stimulation of the rate of cell division after 1 day of cultre and a slight stimulation of cell division of the Friend cells after 2 days of culture. These data indicated that there is not a long lag before H1 histone and polylysine affect cell division and that these compounds are not acting merely by affecting the level of depletion of some material in the culture medium after a prolonged period of culture. DNA fiber autoradiographic experiments revealed that replicon size is decreased in the cells cultured with H1 histone and polylysine at later periods of culture. (25 Refs)

30. SERUM SUBSTITUTES WHICH SUPPORT THE ATTACHMENT AND COLONY OUTGROWTH OF CULTURED LIVER EPITHELIAL CELLS (MEETING ABSTRACT).

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In Vitro; 17(3):237 1981

The effect of hormones and growth factors on proliferation of liver epithelial cells in serum-free media was studied. The cell line used was one of many established in our laboratory and routinely subcultured at weekly intervals in Ham's F-10 medium with 10% fetal bovine serum (FBS). A non-malignant cell line from a newborn Fisher rat was used between passage 10 and 20. When 0.75 mg/ml fetuin was added to Ham's F-10 medium and cells were plated at high or low seeding density, the number of attached cells increased 1.5 to 2-fold over controls plated in 10% FBS, and they grew at a rate comparable with controls. While fibronectin did not increase the attachability of plated cells, it did permit single cells to grow into larger colonies, compared with control. Insulin supported the attachment of cells, but these were not viable. Cells allowed to attach for 24 hr in serum-free medium containing fetuin were grown for 10 days in Ham's F-10 with various concentrations of serum. In medium containing 1% FBS the number of colonies increased up to 3-fold compared with that containing 10% FBS. This shows a reduced requirement of FBS for this cell line. (no Refs)

31. GROWTH CONTROL OF PROSTATIC CARCINOMA CELLS IN SERUM-FREE MEDIA: INTERRELATIONSHIP OF HORMONE RESPONSE, CELL DENSITY, AND NUTRIENT MEDIA.

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Proc Natl Acad Sci USA; 78(9,part2):5673-5676 1981

Two established prostatic carcinoma cell lines have been grown in long-term culture in a defined medium (PFMR-4) free of serum, hormones, or growth factors. Growth of both lines in serum-free medium was population dependent. This cell-density requirement could be replaced by mitomycin C-inactivated feeder cells, homologous conditioned medium, or fetal bovine serum, but not by hormones or growth factors. The cells responded to these factors only at high density. The nature of this hormonal response was dependent on the kind of basal nutrient medium used. Growth in PFMR-4 with added insulin was more rapid than that in DME/F12 medium with any combination of hormones or growth factors and was substantially greater than growth in DME/F12 medium with insulin alone. The results demonstrate that whereas these two prostatic carcinoma lines (PC-3 and DU 145) do not require hormones for survival or growth, they do respond to certain hormones under appropriate conditions. These conditions include

both the type of basal nutrient medium used and the population density. (Author abstract) (20 Refs)

32. GROWTH RESPONSE TO INSULIN AND MSA IN MOUSE MELANOMA CELLS AND FIBROBLAST X MELANOMA HYBRIDS (MEETING ABSTRACT).

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Growth of Cells in Hormonally Defined Media, September 1-4, 1981, Cold Spring Harbor, New York Cold Spring Harbor Laboratory 101 pp., 1981.

Further studies have been performed with the PG19 mouse melanoma cells and one of the insulin-responsive hybrid clones to investigate the biochemical basis for the complementation for the insulin response observed in the hybrids. In hormone-supplemented serum-free medium, the hybrid cells but not the melanoma cells require insulin for optimal growth. Thus the growth response to insulin of the two cell types in serum-free medium is consistant with the growth response observed in confluent growth-arrested cultures. Like insulin, the insulin-like growth factor multiplication stimulating activity (MSA) stimulates 3H-thymidine incorporation in the fibroblast x melanoma hybrid cells but not in the parental melanoma cells. The PG19 melanoma cells and the hybrid cells have approx the same number of high and low affinity insulin receptors, and both cell types also have high affinity MSA receptors. Interestingly, insulin strongly stimulates 3H-leucine incorporation in the hybrid cells over a broad concentration range, while in the melanoma cells it does not have a significant effect in most experiments and has a marginally significant effect in others. Two possible explanations for these results are that the melanoma cells have a postr eceptor block in the growth response to insulin and MSA, or that processes controlled by insulin are never growth-limiting in these cells, whereas such processes can be made growth-limiting in the hybrids. One class of variants appears to arrest more completely in medium with low serum than the parental hybrid cells, and to have a more stringent requirement for serum-derived growth factors for exit from G1. (1 Ref)

33. EFFECT OF TRANSFERRIN ON THE GROWTH OF A HUMAN RECTAL CANCER CELL LINE (MEETING ABSTRACT).

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Gastroenterology; 80(5,part2):1235 1981

Transferrin was examined for its binding properties and effects on growth of a human rectal cancer cell line (HRT-18) in culture. Monolayers were cultured in multiwell plates in media containing 5% fetal bovine serum at 37 C with a 5% CO2 atmosphere and were subjected to binding studies using iodinated transferrin. Effects of transferrin on cell growth were assessed by culturing cells in serum-free synthetic medium in the presence and absence of purified human transferrin at varying concentrations for up to 14 days. Cells were able to grow satisfactorily in a serum-free synthetic medium in the absence of various hormones and growth factors. When included in a serum-free medium, transferrin stimulated cell growth. Doubling x of control cells was 3.5 days, while that of cells grown with 2ug/ml of transferrin was 1.8 days. Iodinated transferrin bound specifically to HRT-18 cells. Equilibrium of binding was attained within 20 min of incubation at 37 C and was shown to be saturable at a concentration of 40nM. Scatchard analysis of the binding data was best explained by a single linear line. The association constant (Ka) was found to be 1.3 x 108M-1 and the number of binding sites per cell was 1.8 x 10(5). In summary: 1) transferrin stimulates the growth of HRT-18 cells, and 2) transferrin specifically binds to HRT-18 cells. These data indicate that transferrin has a growth promoting effect on HRT-18 cells in culture and may affect the growth of some colonic maliignancies. (no Refs)

34. EFFECTS OF HUMAN LYMPHOBLASTOID INTERFERON ON CULTURED BREAST CANCER CELLS.

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Int J Cancer; 28(4):447-453 1981

The effect of human interferon on growth and fibronectin production in a range of cultured breast cancer cell lines has been investigated. The cell lines differ in their sensitivity to interferon's growth-inhibitory effect as determined from growth curves, but the degree of sensitivity varies according to the method of assay. When growth was measured in cultures seeded with fairly large numbers of cells, five out of seven lines examined showed a reduction of 50% or more in the number of attached cells after treatment for 8 days with 1,000 units/ml of interferon. Growth of the two cell lines least affected by interferon treatment in dense culture could, however, be markedly inhibited under conditions of clonal growth in agar or when sparsely seeded on plastic and this was due to inhibition of initiation of growth. In some breast epithelial lines, where the number of attached cells was regulated in part by cell shedding, it was found that interferon not only inhibited cell proliferation, but also increased cell shedding. To determine if interferon increased cell shedding by affecting fibronectin production, the total and cell associated fibronectin was estimated in control and interferon-treated cultures. Four of 10 cell lines examined produced detectable amounts of fibronectin, and in only 1 of these lines, Hs578T derived from a carcinosarcoma, was the production and retention of fibronectin inhibited by interferon treatment. In those breast carcinoma lines which produced detectable amounts of fibronectin, the level of production and retention of the protein, expressed as a proportion of the total protein, did not change after interferon treatment, but showed an increase if expressed on a per cell basis. This is explained by the fact that the interferon-treated cells showed an increase in cell vol. (Author abstract) (36 Refs)

35. INHIBITION OF CELL PROLIFERATION AND LOCOMOTION BY INTERFERON: MEMBRANE AND CYTOSKELETAL CHANGES IN TREATED CELLS (MEETING ABSTRACT).

Tamm I, Pfeffer LM, Wang E, Landsberger FR, Murphy JS
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Thirteenth Miami Winter Symposium: Cellular Responses to Molecular Modulators, January 12-16, 1981, Miami, Florida. University of Miami 112 pp., 1981.

Treatment of human fibroblasts in monolayer culture with human fibroblast interferon (640 u/ml) progressively inhibits the proliferation and locomotion of cells. By the 3rd day after the beginning of treatment, the proliferation rate has decreased to approx 30% of that of control cells and locomotion to approx 20%. The initial doubling time of control cells is approx 18 hr and it increases to approx 33 hr by the 3rd day. Control cells move, on the av, 0.13 um/min throughout the course of an experiment. In interferon-treated fibroblasts which continue to divide, the intermitotic interval becomes progres-

sively prolonged. However about one-half of the offspring of the cells which have an increased first intermitotic interval fail to divide again. In contrast, most of the treated cells which initially have a generation time similar to that of control cells undergo further mitoses. With each succeeding cell generation, the dividing cells represent a progressively smaller fraction of the population, but no abnormal rounding, de generation or lysis of cells occurs. Treatment of HeLa-S3 cells in suspension culture with human fibroblast interferon (640 u/ml) also inhibits the proliferation of cells and increases the rigidity of the plasma membrane lipid bilayer. The ability of treated cells to redistribute cell surface receptors for concanavalin A (Con A) is impaired. Capping of fluorescein-conjugated Con A is inhibited approx 85% 36 hr after beginning of treatment. In interferon-treated cells there is a thick submembranous layer of microfilaments which is not observed in control cells. We conclude that interferon treatment of cells elicits a coordinated response that involves the cell membrane with its associated proteins and the cytoplasmic microfilaments. The response entails early changes as well as changes that may be secondary to the impairment of cell cycling by interferon. (4 Refs)

HORMONES/ STEROIDS/ PROSTAGLANDINS AS GROWTH EFFECTORS

36. GLUCOCORTICOID-MEDIATED INHIBITION OF ORNITHINE DECARBOXYLYASE ACTIVITY IN S49 LYMPHOMA CELLS.

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Proc Natl Acad Sci USA; 78(9,part2):5669-5672 1981

Incubation of wild-type S49 lymphoma cells with glucocorticoids, such as dexamethasone and hydrocortisone, inhibits the activity of ornithine decarboxylase (L-ornithine carboxylyase, EC 4.1.1.17), the rate-limiting enzyme in the pathway of polyamine biosynthesis. The kinetics of this inhibition are more rapid than the glucocorticoid-mediated growth arrest in the G1 phase of the cell cycle or in glucocorticoid-mediated cytolysis of these cells. The inhibition of ornithine decarboxylase activity by corticosteroids is specific for steroids of the glucocorticoid class. Results obtained with variant S49 cells having lesions in the pathways of glucocorticoid or cyclic AMP action indicate that cytoplasmic glucocorticoid receptors, as well as nuclear transfer of steroid-receptor complexes, are required for the inhibition of ornithine decarboxylase activity but that this inhibition does not require hormonal activation of adenylate cyclase or cyclic AMP-dependent protein kinase. Because glucocorticoid-mediated inhibition of ornithine decarboxylase occurs when cellular protein synthesis has decreased less than 20%, this inhibition may represent a specific glucocorticoid-mediated deinduction of ornithine decarboxylase in S49 cells. Inhibition of ornithine decarboxylase activity may offer a useful marker for suppression of growth and cell cycle progression in these and other lymphoma cells. (Author abstract) (33 Refs)

37. EFFECTS OF DEXAMETHASONE ON LIPID METABOLISM IN HUMAN CHRONIC LYMPHATIC LEUKEMIA CELLS (MEETING ABSTRACT).

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In Vitro; 17(3):232 1981

Dexamethasone (dex) causes a 25% inhibition of growth of a human chronic lymphatic leukemia (CLL) cell line following two days of exposure. Although the basis of this growth inhibition is not known, a number of changes in lipid metabolism have been observed in cells exposed to dex. A 35% inhibition of 3H-acetate incorporation into the non-saponifiable lipid fraction of CLL cells was observed after one hr of exposure to dex. Acetate incorporation into the saponifiable lipids was decreased by 20% after two hr of exposure to dex followed by a 20% inhibition of 3H-thymidine incorporation into trichloracetic acid precipitable material after four hr of exposure to steroid. This later resulted in a 40% decrease in total cellular phospholipid content although cellular cholesterol levels remained unaffected. In spite of the decreased phospholipid content, a 24 hr exposure of CLL cells to dex caused a 2-fold stimulation of cytidine diphosphate-choline transferase activity which corresponded with a 25% increase in choline incorporation into phosphatidyl choline. However choline incorporation into sphingomyelin was inhibited by 50%. The resultant alteration in the sphingomyelin/phosphatidyl choline ratio of CLL membranes may be an important factor in the growth inhibition caused by dex. (no Refs)

38. ROLE OF ESTROGENS AND GROWTH FACTORS IN PITUITARY TUMOR CELL GROWTH (MEETING ABSTRACT).

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Growth of Cells in Hormonally Defined Media, September 1-4, 1981, Cold Spring Harbor, New York Cold Spring Harbor Laboratory 101 pp., 1981.

Preliminary evidence suggests that estrogens causes an increase in total protein per cell as measured by flow cytometry methods. Taken together, the data suggest that while estrogens may have an effect on cell metabolism, they do not control complete traverse of the growth cycle. Conversely, estrogen-inducible growth factors derived from rat uteri and kidneys and from rabbit uteri promote continuous growth of GH3/C14 cells in serum-free medium. These observations support our previous proposal that both the uterine and kidney derived activities could act in vivo as sources of protein mediators (estromedins) for estrogen-responsive pituitary cell growth. Efforts to utilize the large scale rabbit uteri and kidney sources for purification are in progress. In other studies we have found no evidence of autocrine/paracrine production of pituitary cell growth factors by normal pituitary gland or GH3/C14 pituitary tumors. Finally, since a considerable amount of neuroendocrine data exists showing the hypothalamus to produce physiology control substances for anterior pituitary, we have prepared extracts of female rat hypothalamus, cerebellum, cerebum and brain stem, and assayed for growth factor activity using the GH3/C14 cells in serum free medium supplemented only with extract proteins. Hypothalamic extract protein, at levels of up to 94 ug/ml, promoted growth of GH3/C14 cells as measured by an increase in cell number, while extracts of other areas of the rat brain showed no activity. (no Refs)

39. DIFFERENT EFFECTS OF OESTRADIOL, OESTRIOL, OESTETROL AND OF OESTRONE ON HUMAN BREAST CANCER CELLS (MCF-7) IN LONG TERM TISSUE CULTURE.

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Acta Endocrinol (Copenh); 98(1):73-80 1981

The effects of estradiol (Oe2), estrone (Oe1), estriol (Oe3), estetrol (Oe4) on the induction of the progesterone receptor (PgR) and growth of MCF-7 cells are compared. All the four estrogens increase cell PgR concentration. Analysis of the dose-response curves shows induction by Oe2 to be 10x and 50x greater than Oe3 and Oe4, respectively. Oe1 and Oe2 are equally effective, even with consideration of metabolic conversion of Oe1 into Oe2. When compared with untreated cells, Oe2, Oe3 and Oe4 do not influence significantly the plating efficiency but all three hormones increase thymidine incorporation of cells in log phase growth. Oe2, Oe3 and Oe4 are able to rescue the growth inhibition induced by antiestrogens. The respective potency compared to Oe2 is again in the range of 10 and 50x lower for Oe3 and Oe4, respectively. On the other hand Oe1 decreases plating efficiency, thymidine incorporation and does not rescue the growth inhibition induced by antiestrogens when the metabolic conversion of Oe1 into Oe2 is prevented. Thus, Oe3 and Oe4 behave like complete Oe2 agonists whereas Oe1 has dissociated effects, agonist on PgR induction and antagonist on cell growth. (Author abstract) (24 Refs)

40. EVIDENCE FOR A NEW ROLE OF PITUITARY DERIVED HORMONES AND GROWTH FACTORS IN MAMMARY TUMOR CELL GROWTH IN VIVO AND IN VITRO (MEETING ABSTRACT).

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Growth of Cells in Hormonally Defined Media, September 1-4, 1981, Cold Spring Harbor, New York Cold Spring Harbor Laboratory 101 pp., 1981.

Evidence has been obtained that estrogen-responsive growth in vivo of the MTW9/PL rat mammary tumor cells can be stimulated 4.3-to 6.6-fold by coimplantation of the growth hormone (GH) and prolactin (PRL) secreting GH3/C14 rat pituitary tumor cells. The GH or PRL secreted by the GH3/C14 tumor could be directly mitogenic for MTW9/PL cells; neither of these hormones was seen to be mitogenic for MTW9/Pl cells over a broad range of concentration. We showed that elevated GH levels induced by the GH3/C14 tumor le ad to 3-to 7-fold elevated levels of somatomedin C (SmC) in W/Fu rats. Thus, we asked whether SmC was mitogenic for MTW9/PL cells; up to 500 ng/ml SmC showed no mitogenic activity. Furthermore, MTW9/PL cells contain very low levels of SmC receptors. Elevated PRL could induce the production of as yet unrecognized factors which are then mitogenic for MTW9/PL cells. This is being directly tested now and preliminary data show that hypophysectomy causes a decrease in MTW9/PL growth factor activity in serum. The GH3/C14 tumors themselves in vivo produce growth factors which promote the growth of the MTW9/PL cells. Extracts of GH3/C14 tumors from estrogen-treated animals were prepared and assayed for mitogenic activity with MTW9/PL cells. These extracts showed a high specific growth factor activity which replaced the serum growth requirement of the MTW9/PL cells. Extracts of normal rat pituitary glands also showed growth factor activity toward MTW9/Pl cells, although the specific activity was 2.7 x less than that of the GH3/C14 tumors. (no Refs)

41. INHIBITION OF PROSTATE TUMOR GROWTH IN TWO RAT MODELS BY CHRONIC ADMINISTRATION OF D-TRP6 ANALOGUE OF LUTEINIZING HORMONE-RELEASING HORMONE.

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Proc Natl Acad Sci USA; 78(10):6509-6512 1981

We have investigated the effect of the D-Trp6 analogue of luteinizing hormone-releasing hormone (LH-RH), a superactive analogue of LH-RH, on the growth of two different models of prostate tumors in rats. Chronic administration of D-Trp(6)-LH-RH in a dose of 25 ug/day for 14-21 days significantly inhibited the growth of the chemically induced squamous cell carcinoma 11095 in Fisher 344 male rats. The wt of the ventral prostate and testes were also significantly reduced by treatment with this analogue. After 21 days of treatment, the animals no longer showed increases in serum luteinizing hormone and follicle-stimulating hormone levels in response to D-Trp(6)-LH-RH. Treatment of male Copenhagen F-1 rats bearing the Dunning 3327 prostate adenocarcinoma with 25 ug of D-Trp(6)-LH-RH per day for 42 days decreased the wt of both the ventral prostate and testes but had no effect on the wt of the anterior pituitary gland. The percentage increase in tumor vol was decreased to one-third and the actual tumor wt was decreased by 58% compared to untreated controls. The tumor doubling time was more than 4x longer in rats receiving D-Trp(6)-LH-RH than in controls. Serum levels of luteinizing hormone and follicle-stimulating hormone were significantly decreased in rats receiving this analogue. In both Fisher 344 and Copenhagen F-1 rats, serum prolactin and testosterone levels were significantly decreased after treatment with D-Trp(6)-LH-RH, whereas progesterone levels were increased. (Author abstract) (34 Refs)

42. A GENETIC APPROACH TO STUDIES OF INSULIN ACTION IN CLOUDMAN S91 MELANOMA CELLS (MEETING ABSTRACT).

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Growth of Cells in Hormonally Defined Media, September 1-4, 1981, Cold Spring Harbor, New York Cold Spring Harbor Laboratory 101 pp., 1981.

Wild-type Cloudman S91 mouse melanoma cells have prolonged generation times when insulin is added to culture medium containing either horse or fetal calf sera. However, in the defined, serum-free medium of Mather and Sato the cells are stimulated to proliferate by insulin. In both cases the effects are mediated through specific high affinity insulin receptors. The effects of insulin on proliferation are dissociable from those on amino acid transport and glycogen synthesis. We isolated a number of mutants which are resistant to the inhibitory effects of insulin in serum-containing medium, and have identified three distinct phenotypes: insulin inhibited (wild-type), insulin resistant, and insulin dependent. In addition, 'revertants' of the insulin dependent line were selected which no longer require insulin for proliferation. Each revertant line expressed the insulin-resistant phenotype. The insulin dependent line was about 15 x more sensitive than the wild-type to the effects of insulin, and is, to our knowledge, the only line described which is stimulated to proliferate by concentrations of insulin in the 0.1-1.0 nM range. In addition, the insulin dependent line exhibited insulin-mediated protein phosphorylation patterns which differed from those seen in wild-type cells. We have produced

hybrids between cells from each of the above four phenotypic categories. By testing the effects of insulin on proliferation of hybrid clones, we were able to determine that insulin inhibition is dominant over both insulin dependence and insulin resistance, and that insulin dependence is dominant over insulin resistance. The results indicate that a detailed genetic analysis of the regulation of insulin action in these cells is feasible. The Cloudman melanoma line represents the first system available for such studies. (1 Ref)

43. DIFFERENTIATION IN CULTURE OF A RAT MAMMARY STEM CELL LINE (MEETING ABSTRACT).

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International Conference on Prostaglandins and Cancer, August 30-September 2, 1981, Washington, DC. Lombardi Cancer Center 75 pp., 1981.

Three main types of mammary epithelial cells are distinguishable in normal mature glands and in carcinogen induced tumors, those lining the alveoli, those lining the ducts and the myoepithelial cells round both ducts and alveoli. We have previously isolated a clonal epithelial stem cell line from a dimethylbenz(a)anthracene-induced rat mammary carcinoma which can give rise to both alveolar-like and myoepithelial-like cells in culture. The former process can be accelerated by a combination of dimethyl sulphoxide (DMSO) and the mammotropic hormones, prolactin, hydrocortisone and insulin. We now report that the artificial inducer DMSO can be replaced by prostaglandin (PG)E1 and to a lesser extent by PGE2 and PGF2alpha in accelerating this differentiation step. The alveolar-like cells also produce fewer tumors than their parent cells when injected into immunodeprived (nu-nu) mice. Thus this process of differentiation which can be regulated in part by PGE1 in vitro is also accompanied by a reduction in neoplastic potential when the cells are tested in vivo. (no Refs)

44. REGRESSION OF HEAD AND NECK CARCINOMA WITH A PROSTAGLANDIN-SYNTHESIS INHIBITOR.

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Arch Otolaryngol; 107(11):658-663 1981

The in vivo administration of a prostaglandin-synthetase inhibitor inhibited or reduced tumor growth in cancer patients and experimental animals. Indomethacin, a prostaglandin-synthetase inhibitor and nonsteroidal anti-inflammatory agent commonly used in the management of arthritic patients, acted as an immune adjuvant by decreasing the production of prostaglandins. Seven cases demonstrated that indomethacin taken in the usually recommended dosages causes regression and stabilization of head and neck cancer. The following factors were also considered in this study: prostaglandin production, a survey of other reports of solid neoplasm response to prostaglandin-synthetase inhibitor administration, drug toxicity, irradiation therapy and metastases, the need for tumor biopsy, and the role that reduction in inflammation plays in tumor shrinkage. (Author abstract) (27 Refs)

45. RELATIONSHIP OF DIFFERENTIATION AND PROSTAGLANDIN PRODUCTION IN HUMAN PROMYELOCYTIC LEUKEMIA (MEETING ABSTRACT).

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International Conference on Prostaglandins and Cancer, August 30-September 2, 1981, Washington, DC. Lombardi Cancer Center 75 pp., 1981.

We have compared HL-60 cells in their ability to produce prostaglandins (PG) from exogenous arachidonic acid (AA) before and after differentiation induced with dimethyl sulfoxide or retinoic acid (RA). Superoxide anion radical production, as a measure of granulocytic maturity, in the untreated, DMSO and RA differentiated cells was 3.6, 17.9, and 32.2 nmol/hr/10(6) cells suggesting that RA induced a greater degree of granulocytic differentiation. Likewise, the percent of mature cells by morphology was greater in the RA treated cells. Cells were incubated with [1-14C] AA for 1 hr at 37 degrees. After differentiati on with DMSO, the cells produced limited amounts of PGE2 and PGD2. In contrast, HL-60 cells differentiated with RA produced large amounts of PGE2 and PGD2. There were less than 2000 cpm in bands corresponding to 6-keto-PGF1alpha, PGF2alpha, thromboxane B2 (TXB2), PGA2 or PGB2. Therefore, HL-60 cells differentiated with RA had a 100-fold greater increase in PGE2 production as compared to untreated HL-60 cells and a 6-fold increase over DMSO differentiated cells. These data indicate that in this human promyelocytic leukemia cell line, PG production is dependent upon maturation. Furthermore, HL-60 cells differentiated with RA produce PG and superoxide anion radical at a greater rate than those differentiated with DMSO demonstrating that it is a more effective inducer. (no Refs)

46. PGE2 INDUCED ANGIOGENESIS (MEETING ABSTRACT).

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International Conference on Prostaglandins and Cancer, August 30-September 2, 1981, Washington, DC. Lombardi Cancer Center 75 pp., 1981.

Tumor-induced angiogenesis is believed to be an essential prerequisite for the successful growth of tumors. In addition to tumors, lymphocytes and macrophages have also been implicated in the induction of neovascular reactions. An association of prostaglandin E2 (PGE2) with angiogenesis is indicated by reports of its release by tumors and its presence in inflammatory reactions. In order to determine the ability of PGE2 to induce angiogenesis in the chorioallantoic membrane (CAM) of eight-day-old chick embryos we used PGE2-containing Elvax-40, a slow release polymer. For comparison with the CAM assay, the same preparations were tested for their ability to induce angiogenesis in the skin and cornea of adult mice. Release rates were determined using tritium-labelled PGE2. A release rate of approx 1-5 ng per day resulted in significantly greater angiogenesis on the CAM than did Elvax pellets with release rates either ten x greater or lesser. The release rate of PGE2 found to be optimal for angiogenesis is similar to those reported for a variety of tumors and activated macrophages. This suggests that PGE2 may be an important component in various neovascular reactions. Experiments in progress are aimed at determining the effect of PGE2 and other prostaglandins on the migration and proliferation of capillary endothelial cells in vitro. (no Refs)

47. EFFECTS OF PROSTAGLANDIN 'A' SERIES ON TUMOR CELLS IN VITRO (MEETING ABSTRACT).

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International Conference on Prostaglandins and Cancer, August 30-September 2, 1981, Washington, DC. Lombardi Cancer Center 75 pp., 1981.

The effectiveness of prostaglandin A1 and prostaglandin A2 (PGA1 and PGA2) and t heir 16,16-dimethyl analogues on growth inhibition in B16 amelanotic melanoma and Lewis Lung carcinoma was evaluated. Significant inhibition of proliferation occurred after 5 days with each PGA tested at concentrations of 1.0, 10, and 25 ug/ml. PGA1 and PGA2 were more effective than their dimethyl analogues. Assay for lactase dehydrogenase and vital dye exclusion testing revealed no cytotoxicity at 24 or 48 hr. Inhibition of proliferation was reversible if exposure to PGA's did not exceed 72 hr. DNA synthesis as measured by 3H-thymidine incorporation was also suppressed by both PGA's and the analogues with PGA2 being the most effective inhibitor. Light microscopy of B16 amelanotic melanoma cell cultures exposed to PGA2 for 4 hr showed cessation of mitosis which resumed to an above normal rate following a 24 hr recovery period without PGA2. It was also shown that PGA2 actively promoted the cytodifferentiation of 3T3-LI fibroblasts into mature adipocytes within 21 days. This conversion and accompanying production of triglycerides was much greater with PGA2 than that produced by insulin controls. (no Refs)

48. ARACHIDONIC ACID AND PROSTAGLANDINS STIMULATE THE GROWTH OF PRIMARY NEONATAL RAT HEPATOCYTES (MEETING ABSTRACT).

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In Vitro; 17(3):258 1981

The release of arachidonic acid (AA) from membrane phospholipids and the consequent increase in prostaglandin (PG) synthesis might constitute a biochemical stimulus-response mechanism common to procedures or agents [such as partial hepatectomy or epidermal growth factor (EGF)] which stimulate liver cell growth. Here we report that exogenously administered AA and, to lesser extent, also 3 of its metabolic derivatives (ie PGA1, PGE1, and PGE2) stimulate the DNA-synthetic and mitotic activities of the hepatocytes in neonatal rat liver primary cultures. Thus, at low concentrations, AA is a more effective growth promoter than EGF at equimolar doses, setting 80% of the hepatocytes into active cycling within 24 hr. At low concentrations, PGA1 was as stimulatory as EGF, whereas PGE1 and PGE2 were by 50% less active than EGF. The present results appear to support the possibility that AA and PG's are involved in the regulation of hepatocyte proliferative activation. However, this contention remains to be proven, because such agents might as well have acted by inducing intracellular surges of known mitogenic agents, like cyclic AMP and guanosine monophosphate. (2 Refs)

CYCLIC NUCLEOTIDE/ POLYAMINES/ NUCLEOTIDES AS GROWTH EFFECTORS

49. INHIBITION OF CELL GROWTH OF HUMAN HEPATOMA CELL LINE BEL-7402 BY DIBUTYRYL CYCLIC AMP AND AMINOPHYLLINE.

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Acta Anat Sin; 12(2):187-191 1981

The effects of dibutyryl cyclic AMP and aminophylline on growth of human hepatoma (BEL-7402) cells and on their ultrastructure are described. When used alone (at 0.2 mM), both compounds inhibited cell growth; the effects increased with increasing concentrations. When used in combination, they had synergistic effects on cell growth. The inhibitory effects of the two compounds, whether used alone or in combination, were reversible. Under the combined action of the two drugs, BEL-7402 cells became elongated, with appearance of granules in the cytoplasm. Ultrastructural changes induced by these compounds included: (1) decrease in number of microvilli on cell surface; (2) increase in number of tonofibrils; and (3) loss of regularity in polyribosome distribution. These changes suggest that the inhibitory effects of dibutyryl cyclic AMP may be a result of its ability in inducing cell differentiation in BEL-7402 cells. (13 Refs) (In CHI)

50. INDUCTION OF THE REGULATORY SUBUNIT OF TYPE I ADENOSINE CYCLIC 3':5'-MONOPHOSPHATE-DEPENDENT PROTEIN KINASE IN DIFFERENTIATED N-18 MOUSE NEUROBLASTOMA CELLS.

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Cancer Res; 41(11,part1):4579-4587 1981

The expression of a cyclic AMP (cAMP)-binding protein, regulatory subunit of the Type I cAMP-dependent protein kinase (RI), and its functional significance in the differentiation of N-18 mouse neuroblastoma cells were examined. 8-Azidoadenosine cyclic 3':5'-[32P]monophosphate, a photoaffinity-labeling analog of cAMP, and high-resolution sodium dodecyl sulfate-polyacrylamide gel electrophoresis were used to identify and quantitate cAMP-binding proteins in cell extracts. The induction of differentiation of N-18 mouse neuroblastoma cells, initiated either by adding dibutyryl cAMP to the growth medium or by culturing cells in medium supplemented with 1% fetal calf serum, led to a three-fold increase in the amount of 8-azidoadenosine cyclic 3':5'-[32P]monophosphate incorporated into RI, when assayed in vitro. This increased incorporation was attributable to an increase in the amount of RI rather than to an increase in the affinity of RI for 8-azidoadenosine cyclic 3':5'-[32P]monophosphate. The subunit mol wt, isoelectric point, and immunoreactivity of RI were found to be identical to that of the regulatory subunit of the Type I cAMP-dependent protein kinase purified from bovine skeletal muscle. The increase in RI was not accompanied by an increase in the cAMP-dependent protein kinase activity. DEAE-cellulose column chromatography confirmed the induction of RI as a free cAMP-binding protein in the differentiated neuroblastoma cells. The possibility of a growth-dependent regulation of RI was also examined. Addition of 2% dimethyl sulfoxide to culture s of N-18 mouse neuroblastoma cells inhibited cell growth without increasing the specific activity of RI. Dimethyl sulfoxide had no effect on neurite outgrowth or acetylcholinesterase

activity, two parameters characteristic of differentiated cells. The fact that the induction of RI coincided with differentiation of the neuroblastoma cells suggests that the expression of RI may be used as a biochemical index of differentiation in these cells. The presence of a free cAMP-binding protein, not associated with cAMP-dependent protein kinase in neuroblastoma cells, raises important considerations concerning the action of cAMP in the regulation of growth and differentiation. (Author abstract) (33 Refs)

51. THE GROWTH OF PRIMARY HUMAN ADRENAL CONN'S ADENOMA CELLS IS HORMONE-DEPENDENT (MEETING ABSTRACT).

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Department of Human Anatomy, University of Padua, I-35100 Padua, Italy
In Vitro; 17(3):235 1981

Human adrenal Conn's adenomatous cells were cultured from aldosterone hypersecreting tumors according to a method used for the normal adrenal tissue. In such cultures the epithelial-like cells, exposed to Eagle's minimal essential medium added with 10% inactivated fetal bovine serum, were grouped in monolayered, homogenous colonies and were capable of spontaneous, though slow, proliferation. To identify the factors that affect the growth of these cells, 16-day-old cultures from 10 adenomata were treated for 5 days with daily doses of one of the following agents: ACTH, angiotensin II (AII), cyclic AMP, cyclic guanosine monophosphate (GMP), or with equimolar mixtures of the two hormones or cyclic nucleotides. When given singly, none of such agents could change the basal growth rate of the adenoma cells. In contrast, the mixture of cyclic AMP and GMP elicited a 3-to 4-fold increase in the fractions of cells entering S and M phases, thus doubling the number of adenoma cells within 5 days. Even the mixture of ACTH and AII promoted adenoma cell proliferation, being however only 60% as effective as the associated cyclic agents. The present results support the view that Conn's adenoma cell growth is hormonally controlled and may be mediated via both cyclic AMP and GMP; they also suggest that some other, as yet unidentified, growth factors(s), besides AII and ACTH, may be involved as well in the proliferative control of this histologically benign tumor. (1 Ref)

52. EFFECTS OF DEOXYNUCLEOSIDES ON CULTURED HUMAN LEUKEMIA CELL GROWTH AND DEOXYNUCLEOTIDE POOLS.

Ross DD, Akman SA, Schrecker AW, Bachur NR
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Cancer Res; 41(11,part1):4493-4498 1981

We investigated the mechanism of cell growth inhibition caused by the deoxyribonucleosides thymidine (dThd), deoxyguanosine (dGuo), deoxyadenosine (dAdo), and deoxycytidine (dCyd). Growth of the cultured human leukemic cells HL-60 and K-562 was measured by cloning in soft agar. Of the deoxyribonucleosides, dGuo was the most potent cell growth inhibitor; however, the potency of added dAdo was probably attenuated by the presence of adenosine deaminase in the tissue culture growth medium. The concentrations of nucleoside causing 50% inhibition of HL-60 cloning were: dCyd, greater than 10,000 uM; dAdo, 500 uM; dThd, 5,000 uM; and dGuo, 80 uM. For K-562 cloning, the concentrations causing 50% inhibition of cloning were dCyd, greater than 10,000 uM; dAdo, 1,600 uM; dThd, 880 uM; and dGuo, 100 uM. Measurement of deoxycytidine 5'-triphosphate (dCTP) pool size in HL-60 cells following incubation with 750 uM deoxyribonucleosides revealed that dGuo caused the greatest reduction of dCTP pools, both in early (passage 10)- and late (passage 71)-passage-derived HL-60 cell cultures (35 and 19% of control, respectively), compared to dThd (61 and 26% of control, respectively) and dAdo (39% of control of HL-60 passage 10). In K-562 cells, reductions in dCTP pool size caused by dAdo, dThd, and dGuo were 67, 46, and 35% of control, respectively. Incorporation of [3H]dCyd into DNA of HL-60 and K-562 cells was enhanced by dThd and dGuo, but the degree of enhancement was greater for dThd than for dGuo. Despite its effect in reducing HL-60 dCTP pool size, dAdo failed to enhance [3H]dCyd incorporation in either HL-60 or K-562 cells. Addition of dCyd to the cultures could only partially rescue the inhibition of HL-60 cloning caused by dThd or dGuo, suggesting that inhibition of cytidine 5'-diphosphate reduction by ribonucleotide reductase is not the only mechanism whereby these nucleosides inhibit leukemic cell cloning. These data suggest that, in addition to inhibiting de novo dCTP production via ribonucleotide reductase, these nucleosides may affect other processes in the salvage pathway such as cellular uptake and phosphorylation or the DNA polymerase reaction itself. (Author abstract) (40 Refs)

COFACTORS/ NUTRITIONAL GROWTH FACTORS

53. 1ALPHA,25-DIHYDROXYVITAMIN D3 INDUCES DIFFERENTIATION OF HUMAN MYELOID LEUKEMIA CELLS.

Miyaura C, Abe E, Kuribayashi T, Tanaka H, Konno K, Nishii Y, Suda T
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Biochem Biophys Res Commun; 102(3):937-943 1981

Human promyelocytic leukemia (HL-60) cells were inoculated at 1 x 10(5) cells/ml on day 0 and incubated for 4 days. Cell suspensions were grown without (control) or with 1.2 x 10(-10), 1.2 x 10(-9), or 1.2 x 10(-8) M 1alpha,25-dihydroxyvitamin D3 (D3). Cell growth was inhibited in a dose- and time-dependent manner; inhibition was effected at 10(-9) M D3 and at 10(-8) M D3 viable cell count was decreased to 20% of control. Addition of 1.2 x 10(-8) M D3 induced 41% of cells to differentiate from promyelocytes with large round nuclei into myeloid cells including myelocytes and metamyelocytes; 3% differentiated into mature granulocytes. Inducing activity of D3 was similar to that found with dimethyl sulfoxide (DMSO) and actinomycin D but not 12-O-tetradecanoyl-phorbol-13-acetate (TPA). Incubation of HL-60 with D3 induced cell adhesion to dish surfaces, phagocytic activity, and rosette formation. With 1.2 x 10(-8) M D3, 53% of cells became phagocytic vs 6.3% of controls; C3 receptor formation was seen in 57% vs 9.5% of control cells. Formation of Fc receptors was not affected by D3. Similar results were obtained with DMSO, actinomycin D, and TPA, except that TPA slightly induced formation of Fc receptors. Results indicate that, like TPA, DMSO, and actinomycin D, D3 at physiological plasma concentrations is involved in cell growth and differentiation of human myeloid leukemia cells. (19 Refs)

54. EFFECTS OF RETINOIDS ON KERATINOCYTES IN VITRO.

Mahrle GO, Wilkinson DI, Farber EM
Universitats-Hautklinik, Von-Siebold-Strasse 3, D-3400 Gottingen, W. Germany
Retinoids: Advances in Basic Research and Therapy, Proceedings of the International Dermatology Symposium held in Berlin, W. Germany, 13-15 October, 1980. Orfanos CE, Braun-Falco O, Farber EM, Grupper C, Polano MK, and Schuppli R, ed. Berlin, Springer-Verlag, 527 pp., 1981.

In tests on guinea pig epidermal cells in vitro vitamin A acid (VAA) stimulated cell growth and protein synthesis and increased the absolute and relative numbers of attached cells during the replicative and proliferative phases. VAA promoted detachment of cells during the plateau phase of cell growth. The aromatic retinoid Ro 10-9359 had little or no such effects. (20 Refs)

55. RETINOIDS: NEW DEVELOPMENTS IN THEIR MECHANISM OF ACTION AS RELATED TO CONTROL OF PROLIFERATIVE DISEASES.

Sporn MB
Lab. Chemoprevention, Div. Cancer Cause and Prevention, NCI, Bethesda, MD
Retinoids: Advances in Basic Research and Therapy, Proceedings of the International Dermatology Symposium held in Berlin, W. Germany, 13-15 October, 1980. Orfanos CE, Braun-Falco O, Farber EM, Grupper C, Polano MK, and Schuppli R, ed. Berlin, Springer-Verlag, 527 pp., 1981.

New evidence suggests that retinoids block proliferation induced by polypeptide hormones (PH) or hormone-like agents. Whether the antiproliferative effects of retinoids on epithelial cell differentiation involves modulation of the action of PH is unknown. Retinoids have been shown to have an antagonistic effect on proliferative PH in several disease states, especially malignancy. (15 Refs)

EXOGENOUS AGENTS AFFECTING DIFFERENTIATION

56. TERMINAL DIFFERENTIATION SURFACE ANTIGENS OF MYELOMONOCYTIC CELLS ARE EXPRESSED IN HUMAN PROMYELOCYTIC LEUKEMIA CELLS (HL60) TREATED WITH CHEMICAL INDUCERS.

Perussia B, Lebman D, Ip S H, Rovera G, Trinchieri G
Wistar Inst. Anatomy and Biology, 36th and Spruce St., Philadelphia, PA, 19104
Blood; 58(4):836-843 1981

The expression of two surface antigens present on the cell membrane of both human granulocytes and monocytes was studied during the process of myelomonocytic differentiation using two monoclonal antibodies (B9.8.1 and B13.4.1). These surface antigens are not present on immature myeloid cells nor on nonmyeloid hematopoietic cells, but can be detected when the cells are terminally differentiated. Among the bone marrow cells, B13.4.1 binds to metamyelocytes and B9.8.1 to metamyelocytes and a fraction (30%) of myelocytes. HL60 human promyelocytic leukemia cells did not react with such monoclonal antibodies. However, when such cells were induced to differentiate in vitro into mature myeloid elements by treatment with retinoic acid or dimethyl sulfoxide, 70%-90% of the differentiated cells expressed both surface antigens. Cell sorting studies on these treated HL60 cells indicated that myelocytes and metamyelocytes were the most immature cells expressing such markers. Expression of the two surface antigens was also observed when HL60 cells were induced to differentiate into monocyte/macrophage cells by treatment with the tumor promoter 12-0-tetradecanoyl-phorbol-13-acetate. Thus, human promyelocytic leukemia cells induced to differentiate in vitro by treatment with specific chemical agents express membrane antigens in the same pattern as normal bone marrow myeloid cells at the corresponding stage of differentiation. (Author abstract) (26 Refs)

57. INDUCTION OF DIFFERENTIATION OF HUMAN PROMYELOCYTIC LEUKEMIA CELLS (HL-60) BY NUCLEOSIDES AND METHOTREXATE.

Bodner AJ, Ting RC, Gallo RC
(c/o Ting). Biotech Res. Lab., 3 Taft Court, Rockville, MD, 20850
JNCI; 67(5):1025-1030 1981

Various purine and pyrimidine analogs and methotrexate were tested to determine whether they induce morphologic and functional myeloid differentiation in HL-60, a human promyelocytic leukemia cell line. Functional maturity was assessed by nitro blue tetrazolium reduction assays. 3-Deazauridine caused nearly all of the cells to differentiate during 6 days of treatment. Pyrazofurin, virazole, puromycin aminonucleoside, and the tricyclic nucleoside 3-amino-1,5-dihydro-5-methyl-1-beta-D-ribofuranosyl 1,4,5, 6,8-pentaazaacenaphthylene induced maturation in 44-64% of the cells, whereas 5-azacytidine, 5-bromo-2'-deoxyuridine, 5-iodo-2'-deoxyuridine, thymidine, and the antimetabolite methotrexate induced maturation in 28-36% of the cells. In terms of effective concentration, the most potent inducer was methotrexate (10(-8) M). The predominant cell types after treatment with all of these compounds were the metamyelocyte and banded neutrophilic granulocyte. (Author abstract) (34 Refs)

58. INDUCTION OF TERMINAL DIFFERENTIATION OF HL-60 AND FRESH LEUKEMIC CELLS BY RETINOIC ACID (MEETING ABSTRACT).

Breitman TR
Laboratory of Tumor Cell Biology, National Cancer Institute, Bethesda, MD, 20205
Growth of Cells in Hormonally Defined Media, September 1-4, 1981, Cold Spring Harbor, New York Cold Spring Harbor Laboratory 101 pp., 1981.

Retinoic acid (RA) is the most potent inducer of granulocytic differentiation of HL-60 inducing maturation at concentrations that are 10(-3) to 10(-6) less than other inducers and at the physiological concentration of approx 10 nM. While continuous exposure to RA is necessary for optimal differentiation, HL-60 cultures exposed to RA for 12 to 24 hr, mature to a marked extent subsequent to resuspension in RA-free growth medium. The extent of RA-induced differentiation is not affected by either nongrowth or by growth-inhibiting conditions including: treatment of the cultures with the DNA synthesis inhibitors arabinosylcytosine or hydroxyurea and omission of insulin or transferrin from a defined serum-free medium. Differentiation of HL-60 is also induced by prostaglandins E1 and E2 (PGE). Combinations of PGE and RA are markedly synergistic. The results with PGE raised the possibility that RA-induced differentiation of HL-60 was mediated by endogenous PGE synthesis. However, indomethacin and aspirin, two potent inhibitors of PG synthesis, do not inhibit RA-induced differentiation. These results suggest that, in vivo, PGE synthesized by monocytes may play a role in combination with RA

in granulopoiesis. The findings with HL-60 prompted an investigation of the sensitivity to RA of fresh human myelocytic leukemia cells in primary suspension culture. Of the 25 nonlymphocytic leukemic specimens, only cells from the 4 patients with acute promyelocytic leukemia differentiated in response to RA. (no Refs)

59. PROCAINE INHIBITS THE ERYTHROID DIFFERENTIATION OF MEL CELLS BY BLOCKING COMMITMENT: POSSIBLE INVOLVEMENT OF CALCIUM METABOLISM.

Tsiftsoglou AS, Mitrani AA, Housman DE
Dept. Medicine Hematology-Oncology, Harvard Medical Sch., Beth Israel Hosp., 330 Brookline Ave., Boston, MA, 02115
J Cell Physiol; 108(3):327-335 1981

The ability of the calcium ionophore A23817 or Ca++ to overcome the inhibitory effects of procaine (PC) on commitment of murine erythroleukemia (MEL) cells to dimethylsulfoxide (DMSO)-induced terminal erythroid differentiation was studied. Pretreatment of MEL cells with PC inhibited the proportion of committed cells by 90%, while only 20-30% inhibition was obtained with simultaneous exposure to DMSO. However, addition of PC 24 hr after introduction of DMSO caused almost no inhibition and imposed kinetics i dentical to that of the DMSO control. While pretreatment of MEL cells with PC for 2 hr resulted in a slight inhibition of commitment (46%), more pronounced inhibition (88-94%) was obtained in those cultures pretreated for 6-14 hr. Treatment of ME cells with 1 x 10(-4), 7 x 10(-4), and 1 x 10(-3) M PC 24 hr prior to the addition of 1.5% DMSO resulted in 60, 74, and 80% inhibition of commitment, respectively. Pretreatment of MEL cells with PC prior to addition of DMSO protected these cells from DMSO-induced limitation of proliferation due to commitment. In contrast, cells plated in DMSO in the absence of PC stopped multiplying after approx five generations. PC alone did not reduce the rate of cell division of MEL cells. PC also blocked commitment induced by 6-thioguanine and dimethylformamide. The PC block was reversible; cells reentered the differentiation program without recapitulation of the latent period. Concentrations of Ca++ as low as 0.5 milliM were effective in reversing the PC block. Addition of ionophore A23817 in DMSO/PC-treated cells caused an immediate commitment of these cells. The results indicated that PC blocsa lkssibly Ca++-dependent process in the developmental program which occurs prior to the commitment event. (21 Refs)

60. THE RESPONSE OF CANCER PATIENTS' LYMPHOCYTES TO MITOGEN. THE EFFECT OF INDOMETHACIN ON ADHERENT AND NON-ADHERENT CELLS' ACTIVITY.

Pituch-Noworolska A
Dept. Microbiology and Clinical Immunology, Inst. Pediatrics, Wielicka 265, 30-663 Cracow, Poland
Arch Immunol Ther Exp (Warsz); 29(2):161-165 1981

Lymphocytes from 13 patients (age 28-83) with Stage III or IV breast, stomach, and colon cancer and lymphocytes from 13 normal subjects (age 28-42 yr) were tested for response to concanavalin A (Con A) in the presence or absence of indomethacin (ID). ID had no effect on the poor mitogen response of patient nonadherent cells (NAC) cultured with autologous adherent cells (AC). However, it caused a significant increase in the response of normal NAC cultured with autologous AC. Responses of normal NAC cultured with patient AC also increased in the presence of ID; this suggested that prostaglandins (PG) were not responsible for the depressed Con A response of patient lymphocytes and that patient AC were not defective in ability to produce PG. (16 Refs)

OTHER RELATED STUDIES

61. CLONAL PROLIFERATION OF CULTURED NONMALIGNANT AND MALIGNANT HUMAN BREAST EPITHELIA.

Smith HS, Lan S, Ceriani R, Hackett AJ, Stampfer MR
Dept. Surgery, Albert Einstein Coll. Medicine, Bronx, NY, 10461
Cancer Res; 41(11,part1):4637-4643 1981

We have developed a method for clonal growth of human mammary epithelial cells of both nonmalignant and malignant origin. Plating efficiencies of 1 to 50% were obtained by seeding second-passage mammary epithelial cells on fibroblast feeder layers in an enriched medium composed of various hormones and growth factors, as well as conditioned media from three specific human cell lines. Single mammary epithelial cells seeded sparsely onto the fibroblasts underwent at least eight population doublings to form large, readily visible colonies. Optimal colony formation required both feeder cells and the enriched medium. Epithelial colonies containing at least 16 cells were visible 5 days postseeding, and these colonies continued to grow progressively. Plating efficiency and colony size were similar on UV-irradiated or nonirradiated fibroblasts. The number of colonies formed was proportional to the number of epithelial cells plated. The colonies were identified as epithelial by the presence of human mammary epithelial antigens. (Author abstract) (31 Refs)

62. PROTEIN-FREE MEDIUM FOR C-1300 MOUSE NEUROBLASTOMA CELLS.

Agy PC, Shipley GD, Ham RG
(c/o Ham) Dept. Molecular, Cellular and Developmental Biology, Univ. Colorado, Boulder, CO, 80309
In Vitro; 17(8):671-680 1981

An optimized medium, designated MCDB 411, has been developed for mouse neuroblastoma cells. At cell densities of 1 x 10(4) cells/cm2 or greater, several different clones of C1300 mouse neuroblastoma cells can be cultured serially in Medium MCDB 411 with no serum or other undefined supplementation and with a doubling time of about 24 hr. At clonal densities it is necessary to supplement the medium with 1.0 ug/ml insulin. Alternately, good clonal growth can be obtained without direct supplementation by coating the culture dishes with insulin and rinsing off all that is not tightly bound. Primary cultures of cells from serially transplanted C1300 tumors that have never been cultured previously in vitro can be established directly in unsupplemented Medium MCDB 411 with rapid initiation of multiplication and no apparent crisis or selection for minority cell types. The availability of a synthetic medium that supports growth of neuroblastoma cells without supplementation should facilitate the use of these cells as model systems for the study of neuronal function and differentiation. (Author abstract) (26 Refs)

63. MUTUAL INFLUENCE OF CELL DENSITY AND SERUM-FREE NUTRIENT MEDIA ON HORMONAL GROWTH STIMULATION IN PROSTATIC CARCINOMA CELLS (MEETING ABSTRACT).

Kirk D, Szalay M, Vener G, Kaighn ME
Pasadena Foundation for Medical Research, Pasadena, CA, 91101
In Vitro; 17(3):233 1981

The objective of this study was to determine the hormonal requirements of PC-3, a human prostatic carcinoma line, in serum-free medium. Growth of PC-3SF12, a subline of PC-3, at both clonal and high density was compared in DME/F12 or PFMR-4 alone, or with added hormones (insulin, transferrin, T3, thyroid releasing factor, luteinizing hormone releasing factor, luteinizing hormone, fibroblast gr owth factor). At clonal density, no growth was observed in either nutrient mixture with or without hormones. When cells were plated at high density, they grew in both DME/F12 and PFMR-4. Moreover, at high density the cells responded to hormones differently in each medium. Growth stimulation in DME/F12 was largely due to insulin and transferrin. Further stimulation by luteinizing hormone and luteinizing hormone releasing factor was seen. Only insulin increased growth in PFMR-4. It was concluded that: (a) PC-3SF12 has not absolute hormonal requirement, (b) both serum-free growth and hormone responsiveness are population-dependent and (c) the hormone response depends upon the nutrient medium. These results suggest that the hormonal reponse seen in high density culture represents a synergistic interaction of the hormone with a cell product. (no Refs)

64. PHENOTYPIC CHANGE OF AN SV40-TRANSFORMED MOUSE MACROPHAGE LINE, BB-W-531-2, INDUCED BY DIFFERENT CULTURAL METHODS.

Takayama H, Tanigawa T, Takagi A
Dept. Bacteriology, Tottori Univ. Sch. Medicine, Yonago, Tottori 683, Japan
Microbiol Immunol; 25(8):827-835 1981

The effect of culture methods on the characteristics of Simian virus 40 (SV40)-transformed macrophages, BB-W-531-2, established from BALB/cAN mouse bone marrow cells was studied. Results indicated that cell growth and differentiated cell properties of clonal macrophages (BB-W-531-2 line) are controlled by culture methods. The cells passaged by the first method showed a reversible transition between the macrophage and non-macrophage states depending upon cell density. The cell passaged by the second method retained their macrophage properties throughout the culture and grew very slowly. The cells passaged by the third method did not show density-dependent transition. Macrophage properties, such as immune phagocytosis, seemed to be induced by diffusible factor(s) produced by cells possessing such properties. The fact that several properties of BB-W-531-2 cells can be controlled by the culture methods described above suggested the potential value of such control in studies of gene expression regulation. BB-W-531-2 cell growth was correlated with the intensity of SV40 T-antigen and seemed to be suppressed in poor-spreading conditions such as confluent cell density or cultivation on bacteriologic dishes. The development of macrophage properties in cultures of confluent density suggested that both density-dependent grow inhibition and diffusible factors are probably required for induction of macrophage properties. (20 Refs)

65. IN VITRO CELL GROWTH IN NEONATES WITH DOWN'S SYNDROME AND TRANSIENT MYELOPROLIFERATIVE DISORDER.

Denegri JF, Rogers PC, Chan KW, Sadoway J, Thomas JW
Dept. Pathology, Vancouver General Hosp., 855 West 12th Ave., Vancouver, British Columbia, V5Z 1M9, Canada
Blood; 58(4):675-677 1981

Bone marrow and peripheral blood cells from three newborns with Down's syndrome and transient myeloproliferative disorders were cultured in vitro. In the methylcellulose semiliquid system, normal colony formation with maturation and differentiation into granulocytes and monocyte-macrophages were observed in all three patients. This is different from the growth pattern usually seen in acute nonlymphocytic leukemia. A maternal serum inhibitor of both normal allogeneic GM-CFU and blast cell growth was also demonstrated, but its role in pathogenesis is uncertain. Normal in vitro granulopoiesis may help to differentiate the transient myeloproliferative syndrome from congenital leukemia in newborns with Down's syndrome. (Author abstract) (13 Refs)

66. ROLE OF PHOSPHOETHANOLAMINE, ETHANOLAMINE AND PROLACTIN ON MAMMARY CELL GROWTH (MEETING ABSTRACT).

Kano-Sueoka T, Errick JE
Department of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder, CO
Growth of Cells in Hormonally Defined Media, September 1-4, 1981, Cold Spring Harbor, New York Cold Spring Harbor Laboratory 101 pp., 1981.

Phosphoethanolamine (PEtn) and ethanolamine (Etn) are potent growth promoting material for cell lines isolated from a highly hormone-dependent rat mammary carcinoma. Growth stimulation by PEtn or Etn is observed whether the cells are grown in a serum-containing medium or in a serum-free medium containing a mixture of growth factors and hormones. These compounds are taken up by the cells efficiently and literally 100 per cent of those taken up become incorporated into phosphatidylethanolamine of cellular lipid. The phospholipid composition of the cells changes significantly when the growth medium is supplemented with Etn (5uM). The relative amount of phosphatidylethanolamine is three-fold higher in cells grown in the presence of Etn as compared to those grown in the absence of Etn. The proportion of phosphatidylcholine decreases in parallel to the increase in phosphatidylethanolamine. Cell lines isolated from hormone-independent mammary tumor do not respond to PEtn or Etn to grow and, interestingly, the cellular phospholipid composition is not altered whether Etn is present in the medium or not. Growth of the cell line from hormone-dependent tumor is stimulated not only by Etn or PEtn but also by ovine prolactin when the cells are grown in a serum-free medium. The additive effect of Etn and prolactin has also been demonstrated. (no Refs)

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CELL AND SERUM DERIVED GROWTH EFFECTORS

1. EVIDENCE THAT VIRAL TRANSFORMING GENE PRODUCTS AND EPIDERMAL GROWTH FACTOR STIMULATE PHOSPHORYLATION OF THE SAME CELLULAR PROTEIN WITH SIMILAR SPECIFICITY.

Erikson E, Shealy DJ, Erikson RL
Dept. Pathology, Univ. Colorado Sch. Medicine, Denver, CO, 80262
J Biol Chem; 256(22):11381-11384 1981

Treatment of A-431 human epidermoid carcinoma cells with epidermal growth factor (EGF) was shown to enhance the phosphorylation of a Mr = 34,000 protein. Because the phosphorylation of an analogous protein is enhanced in various cell lines transformed by Rous sarcoma virus (RSV) we character-

ized the phosphorylation of the A-431 Mr = 34,000 protein under these two conditions in order to determine wheth er there are common pathways between viral transformation and EGF stimulation. The results of tryptic phosphopeptide mapping and phosphoamino acid analysis showed that the Mr = 34,000 protein was phosphorylated in an identical manner by the EGF-stimulated protein kinase activity and by the protein kinase activity of the RSV transformation-specific protein or of its normal cell homolog. Although the specific protein kinase that phosphorylates the Mr = 34,000 protein under conditions of EGF-stimulation is not yet identified, these studies demonstrate that at least one consequence of EGF stimulation is identical with one of the consequences of viral transformation. (Author abstract) (43 Refs)

2. EPIDERMAL GROWTH FACTOR: I. MORPHOLOGICAL EFFECTS ON HUMAN CARCINOMA CELLS. II. RELATEDNESS OF THE EGF RECEPTOR-KINASE TO A VIRAL TRANSFORMING PROTEIN.

Chinkers MD
Vanderbilt University, Nashville, TN, 37203
Diss Abstr Int [B]; 42(4):1272-B 1981

This dissertation is divided into two parts, each concerned with the interaction of epidermal growth factor (EFG) with A-431 human epidermoid carcinoma cells. EGF induces rapid and dramatic shape changes in these cells; the exact nature of these changes depends on whether Ca++ is present in the medium. After several hr, as cells retracting at the edges of colonies pile up on top of each other, multilayered colonies are formed. This multilayered morphology persists for several days in the presence of EGF, but is freely reversed upon removal of EGF from the medium. The second part of the dissertation concerns the interaction of affinity-purified EGF receptor, prepared from A-431 cells, with antibodies to the transforming protein, pp60src, of Rous sarcoma virus (RSV). This study was initiated, in part, because (a) the EGF-induced morphological changes described above are similar to RSV-induced cell shape changes and (b) both pp60src and the partially purified EGF receptor possess tyrosine-specific protein kinase activity, the latter being stimulated by EFG. The EGF-stimulated kinase, like pp60src, specifically phosphorylates the heavy chain of anti-pp60src IgG; this phosphorylation occurs on tryosine and is stimulated by EGF. However, neither the receptor nor its associated kinase activity are precipitated by these antisera, even those able to precipitate the normal cellular homologue of pp60src (pp60sarc). These results suggest that while the EGF-stimulated kinase is probably not pp60sarc, it may be related to that enzyme. (no Refs)

3. RESPONSIVENESS OF FETAL RAT BRAIN CELLS TO GLIA MATURATION FACTOR DURING NEOPLASTIC TRANSFORMATION IN CELL CULTURE.

Haugen A, Laerum OD, Bock E
Lab. Experimental Pathology, Div. Cancer Cause and Prevention, NIH, Bethesda, MD, 20205
Acta Pathol Microbiol Scand [A]; 89(5):393-402 1981

The effect of partially purified extracts from adult pig brains containing a gli a maturation protein factor (BE) has been investigated on neural cells during carcinogenesis. Pregnant BD IX-rats were given a single transplacental dose of the carcinogen ethylnitrosourea (EtNU) on the 18th day of gestation. The brains of the treated fetuses were transferred to cell culture and underwent neoplastic transformation with a characteristic sequence of phenotypic alterations which could be divided into five different stages. During the first 40 days after explanation (Stage I and II) BE induced morphological differentiation of epithelioid neural cells into astrocytes. this occurred in carcinogen treated cells as well as in untreated control cultures. At the same time cells with astrocyte morphology showed accumulation of glial fibrillary acidic protein (GFA) as tested by indirect immunofluorescence with monospecific antibodies against GFA. Thereafter, in the EtNU pre-treated cultures an increased number of cells with astrocyte morphology was seen, and BE further increased the number of cells with long cytoplasmic processes. Control cells were GFA negative, while some few strongly, as well as many weakly, positive cells were seen after treatment with BE (Stage III). At the later stages of neoplastic transformation the effect of BE became gradually less, and in tumorigenic cells which occurred after about 200-300 days, only a slight morphological change took place in a few cell lines. No appreciable effect on GFA-content was seen any longer, although some few weakly GFA positive cells could be observed in all permanent cell lines. Fetal rat brain cells therefore seem to become less responsive to this differentiation inducer during neoplastic transformation in cell culture. (Author abstract) (36 Refs)

4. GLIAL CELL GROWTH-PROMOTING FACTOR IN ASTROCYTOMA (C6) CELL EXTRACTS.

Kato T, Yamakawa Y, Sakazaki Y, Ito J, Kato H, Tsunooka H, Masaoka A, Tanaka R
Dept. Biochemistry (1st Div.), Sch. Medicine, Nagoya City Univ., Mizuho-ku, Nagoya 467, Japan
Dev Brain Res; 2(4):596-601 1981

The ability of a glia maturation factor (GMF) from rat astrocytoma (C6) cells to stimulate cell proliferation was tested in confluent cultures of a homogeneous population of (methyl-3H)thymidine-labeled glioblasts obtained from rat fetus brain. Exposure of glioblasts to cell extracts containing GMF increased DNA synthesis a max of 300%. Many of the cells rounded up and extruded processes which were branched and interconnected between cells as characterized in mature astrocytes. Cells in control flasks without extract remained flat without such morphological changes. GMF stimulated a 160% increase in number of glioblasts; C6 cells were not stimulated. DNA synthesis of neuroblastoma cells (Neuro-2a) was inhibited in a dose-dependent manner by C6 extracts without decrease in viability or change in neuronal differentiation. Ra t astrocytoma cells appear to contain a factor which affects glioblasts and C6 cells in a manner similar to that of GMF. The factor has not been well characterized physico-chemically. (11 Refs)

5. NERVE GROWTH FACTOR-MEDIATED STIMULATION OF TYROSINE HYDROXYLASE ACTIVITY IN A CLONAL RAT PHEOCHROMOCYTOMA CELL LINE.

Hatanaka H
Dept. Neurochemistry, Mitsubishi-Kasei Inst. Life Sciences, 11 Minamiooya, Machidashi, Tokyo 194, Japan
Brain Res; 222(2):225-233 1981

A subclone of pheochromocytoma cell line PC12 exhibited a significant increase in tyrosine hydroxylase activity over the parental line in the presence of physiologic concentrations of nerve growth factor. It is thought that this new line will be a better tool for elucidating the molecular mechanisms of nerve growth factor action. (27 Refs)

6. PROPERTIES OF A SARCOMA-GROWTH-FACTOR-LIKE PEPTIDE FROM CELLS TRANSFORMED BY A TEMPERATURE-SENSITIVE SARCOMA VIRUS.

De Larco JE, Preston YA, Todaro GJ
Lab. Viral Carcinogenesis, NCI, NIH, Bethesda, MD, 20205
J Cell Physiol; 109(1):143-152 1981

Peptides resembling sarcoma growth factor (SGF) that were produced by virally transformed cells in vitro were characterized. Normal rat kidney (NRK) cells and 3T3 mouse cells were transformed by Moloney sarcoma virus (MSV) or Kirsten sarcoma virus (KiSV). SGF-like factors were obtained from serum-free conditioned media from the cell cultures. Nontransformed cells produced no such factors. The major activity, regardless of cell species or virus type, was produced by a factor with an apparent mol wt of 10,000. Carboxymethyl cellulose chromatography produced similar elution profiles in all cases. Growth factor produced by NRK cells transformed by wild-type KiSV had similar temperature sensitivity to growth factor produced by NRK cells transformed by a temperature sensitive mutant of KiSV. Neither growth factor was temperature-sensitive under the conditions of the assay. THE SGF-like factors produced by transformed cells are apparently produced by cellular genes that are not expressed in the absence of virus. (21 Refs)

7. PROMOTION OF FIBROSARCOMA CELL GROWTH BY PRODUCTS OF SYNGENEIC HOST MACROPHAGES.

Currie GA
Div. Tumor Immunology, Chester Beatty Res. Inst., Belmont, Sutton, Surrey, England
Br J Cancer; 44(4):506-513 1981

Cells from a C57BL/cbi chemically induced fibrosarcoma (FS6) require exogenous platelet-derived growth factor (PDGF) for in vitro proliferation (as do normal 'untransformed' fibroblasts) whereas cells obtained from the FS6M1 tumor, a spontaneous metastasizing subline, show autonomy from PDGF in vitro. furthermore, the FS6 cells exhibit very low colony formation in an anchorage-independent growth assay. In vivo, this tumor is immunogenic, rarely metastasizes and is heavily infiltrated by host macrophages. Studies of in vitro cell proliferation and anchorage-independent growth show that syngeneic host macrophages from the peritoneal cavity or from the growing tumor release a diffusible factor(s) which has (1) growth-stimulating activity on FS6 cells in monolayer cultures in PDGF-poor medium and (2) potent colony-stimulating activity on FS6 cells cultured in methyl-cellulose-containing medium. These macrophage supernatants stimulate proliferation of quiescent normal fibroblasts in monolayer culture as well as FS6 sarcoma cells, but do not stimulate anchorage-independent growth of normal cells. Supernatants from BCG-elicited macrophages were shown to contain abundant arginase, and were cytolytic to FS6 cells but not to normal cells. Heat inactivation abrogated the arginase and cytotoxicity, revealing heat-stable mitogenicity for FS6 cells and normal fibroblasts. The stimulatory effect of macrophages on FS6 sarcoma cells can be mimicked by the addition of the tumor promoter 12-tetradecanoyl-phorbol-13-acetate (TPA) and supports the hypothesis that macrophages could play a significant role in multistage carcinogenesis by providing a source of endogenous promoter. (Author abstract) (20 Refs)

8. TRANSFORMING GROWTH FACTORS (TGFS): PROPERTIES AND POSSIBLE MECHANISMS OF ACTION.

Todaro GJ, De Larco JE, Fryling C, Johnson PA, Sporn MB
Lab. Viral Carcinogenesis, NCI, NIH, Bethesda, MD, 20205
J Supramol Struct Cell Biochem; 15(3):287-301 1981

Properties and mechanisms of action of transforming growth factors (TGF) are described. TGF are mitogenic polypeptides that induce phenotypic changes and promote anchorage-independent growth of normal cells. Several human and animal carcinoma and sarcoma cell lines have been found to produce TGF. Sarcoma growth factor (SGF) is a TGF produced by cell transformed by murine sarcoma virus. TGF bind to cell membrane receptors for epidermal growth factor (EGF), but do not bind antibodies against EGF. biological activity of TGF is different from that of EGF. TGF are not released into culture medium by cells having large numbers of EGF receptors. SGF promotes tumor growth in vitro. The activity of SGF on phenotype is blocked by retinoids. Normal cells exposed to TGF assume the phenotype of transformed cells that produce TGF. Transformed cells may release into culture medium TGF that then bind to their own cell membrane receptors, a process for which the term 'autocrine secretion' (AS) is proposed. The concept of AS may help to explain some aspects of tumor development. (48 Refs)

9. EXTRACELLULAR REGULATION OF FIBROBLAST MULTIPLICATION: A DIRECT KINETIC APPROACH TO ANALYSIS OF ROLE OF LOW MOLECULAR WEIGHT NUTRIENTS AND SERUM GROWTH FACTORS.

McKeehan WL, McKeehan KA
W. Alton Jones Cell Science Center, Lake Placid, NY, 12946
J Supramol Struct Cell Biochem; 15(1):83-110 1981

Relationships among serum-derived growth factors (SGF), nutrients, and survival and multiplication rates were analyzed quantitatively in human fibroblast cultures by enzyme kinetic analysis. Response of cell populations to SGF or nutrients indicated a dissociable, saturable interaction between cells and ligands. Optimization of nutrient concentrations produced max multiplication of normal cells in the presence of SGF and supported maintenance of not multiplying cells in the absence of SGF, indicating that SGF were required for, and played a regulatory role in cell proliferation. Of 29 nutrients 18 were required in larger amounts for multiplication than for cell maintenance. SGF controlled requirements for Ca++, Mg++, Pi, K+, and 2-oxocarboxylic acid for cell multiplication. Transformed cells required smaller amounts of nutrients than normal cells at given levels of SGF. Results suggest that control of cell multiplication is related to external nutrient levels, and that transformed cells may have a nutritional growth advantage. (12 Refs)

10. STIMULATIVE EFFECT OF SERUM ON THE GROWTH OF HELA CELLS SUPPRESSED IN A K+-DEFICIENT CHEMICALLY DEFINED MEDIUM.

Sakai T, Ikehara T, Miyamoto H, Kanilke K
Dept. Physiology, Kinki Univ. Sch. Medicine, Sayamacho, Osaka, 589, Japan
Life Sci; 29(23):2429-2436 1981

Growth of HeLa cells cultured with a chemically defined medium was slightly stimulated in the presence of 5% dialyzed calf serum. The growth-promoting action of serum was more conspicuous when cell growth was suppressed in the same

medium, in which K+ was replaced by Rb+ to various ratios. The growth-promoting factor(s) of serum was heat-labile. Upon addition of dialyzed serum, passive K+ or Rb+ influx was increased, whereas the active cation uptake was unaffected and cell K+ was rather decreased. The serum did not alter uptake of [3H] amino acids. Also, protein synthesis inhibited in the Rb+ substituted medium was not stimulated significantly, except that observed only when the external K+/Rb+ ratio was 1/5. From the distinct effects of serum on cell growth and protein synthesis, we conclude that (i) the serum-induced stimulation of cell growth, which is suppressed in the Rb+ substituted medium, is not a result of the direct effect of serum on synthesis of bulk protein, but a reflection of the effect on another mechanism(s) required for cell growth; and that (ii) this action is basically identical with the growth-promoting action on cells cultured in the normal medium. (Author abstract) (25 Refs)

11. ANGIOGENIC ACTIVITY AND TUMOR.

Miguez M, Davel L, Sacerdote de Lustig E

Instituto de Oncologia Angel H. Roffo, Facultad de Medicina, Av. San Martin 5481, 1417 Buenos Aires, Argentina

Medicina (B Aires); 41(3):369-372 1981

The history of tumoral angiogenic factor (TAF) was presented. TAF separation, bi ochemical characterization, bioassays, angiogenic activity of different origin, TAF usefulness in early diagnosis, and its inhibitors were presented and discussed. (28 Refs) (In SPA)

12. GROWTH OF HUMAN NORMAL AND LEUKEMIC T CELLS: T-CELL GROWTH FACTOR (TCGF) AND THE ISOLATION OF A NEW CLASS OF RNA TUMOR VIRUSES (HTLV).

Gallo RC, De The, Lajtha L, Van Bekkum DW, McCulloch EA, Greaves MJ, Seligmann M, Brecher G, Shohet SB, Bessis M, Sachs L

Lab. Tumor Cell Biology, NCI, NIH, Bethesda, MD, 20205

Blood Cells; 7(2):313-329 1981

Previous studies have indicated that T-cell growth factor (TCGF) is the physiological growth promoter in the immune response of T-lymphocytes, acting as the second signal (after antigen) in the immune response. A summary is presented of information on proliferation of normal T-cells in a previously described liquid suspension culture and proliferation of several new cell lines from patients with particular types of T-cell leukemias and lymphomas. Information on the new type-C virus designated HTLV is also summarized. Neoplastic T cells from patients with cutaneous T-cell leukemias and and lymphomas, eg Sezary syndrome and mycosis fungoides, were grown routinely with the use of purified TCGF free of phytohemagglutinin and of lymphokines other than TCGF. The neoplastic T cells responded directly to TCGF without prior in vitro activation with antigen or lectin as is necessary with normal T cells. Thus, transformed mature T cells already express TCGF receptors. HTLV were isolated from some of these new cell lines. Evidence is presented to indicate these may be the first unambiguous isolates of RNA tumor viruses from humans. These new viruses are easily distinguished from all previously isolated viruses from animals. Antibodies in sera of some patients with these diseases specifically react with proteins of these viruses. (11 Refs)

13. HUMAN CUTANEOUS T CELL LYMPHOMA AND LEUKEMIA CELL LINES PRODUCE AND RESPOND TO T CELL GROWTH FACTOR.

Gootenberg JE, Ruscetti FW, Mier JW, Gazdar A, Gallo RC
Lab. Tumor Cell Biology, NCI, Bethesda, MD, 20205
J Exp Med; 154(5):1403-1418 1981

Constitutive production of T cell growth factor (TCGF) was found in three cell lines derived from mature T cells of patients with cutaneous T cell lymphoma-leukemias (CTCL). TCGF produced by tumor cells (L-TCGF) was found in the culture medium and also bound to the cell membrane. TCGF produced by normal peripheral blood leukocytes (N-TCGF) was adsorbed by normal T lymphocytes that had been activated by phytohemagglutinin (PHA) and by the three CTLC cell lines. N-TCGF was not adsorbed by nonactivated normal T lymphocytes or by other human leukemic cell types. TCGF was eluted from the cell membranes of CTLC and PHA-activated normal T cells using an acid glycine buffer, but could not be eluted from nonactivated cells. T CGF stimulated the growth of activated normal T cells, TCGF-dependent CTCL cells, and TCGF-independent CTCL cells. L-TCGF and N-TCGF had the same biological activity. Production of and response to TCGF by the same cells (autostimulation) apparently is characteristic of some, but not all neoplastic cell populations and enhances proliferation of such cells. (30 Refs)

14. SPONTANEOUS RELEASE OF A FACTOR WITH PROPERTIES OF T CELL GROWTH FACTOR FROM A CONTINUOUS LINE OF PRIMATE TUMOR T CELLS.

Rabin H, Hopkins RF, Ruscetti FW, Neubauer RH, Brown RL, Kawakami TG
Biological Carcinogenesis Program, Frederick Cancer Res. Center, Frederick, MD, 21701
J Immunol; 127(5):1852-1856 1981

A continuous lymphoid cell line had been previously established from a gibbon with spontaneous lymphosarcoma. This cell line, designated as MLA144, when tested after several yr in culture was shown to release spontaneously a factor biologically and biochemically similar to human T cell growth factor (TCGF). Conditioned media (CM) from MLA144 cells support growth and DNA synthesis of T cells from humans, several other species of primates, and also from mice and rabbits. The activity in the MLA144 CM is resistant to 60 C and to low and high pH, has a mol wt, as determined by gel filtration, of 21,500, elutes from DEAE-cellulose at 0.04 to 0.06 M sodium phosphate buffer, pH 7.6, and has an isoelectric point of about 6.45. Surface-marker analysis of MLA144 cells by rosetting techniques indicates that they are T cells lacking in the receptor for the Fc portion of IgG. The release of TCGF by MLA144 cells should have practical value in terms of ease of TCGF production and should be of great help in the facilitation of studies on the cell biology and molecular biology of TCGF production. (Author abstract) (19 Refs)

15. T CELL GROWTH FACTOR RECEPTORS. QUANTITATION, SPECIFICITY, AND BIOLOGICAL RELEVANCE.

Robb RJ, Munck A, Smith KA
Immunology Program, Norris Cotton Cancer Center, Hanover, NH, 03755
J Exp Med; 154(5):1455-1474 1981

T cell growth factor (TCGF) interactions were studied in several cell lines. TCGF bound specifically to receptor sites on activated T cells, producing proliferative response. Unstimulat-

ed murine or human lymphocytes and lipopolysaccharide-activated B cell blasts expressed few if any detectable binding sites. Lectin- or alloantigen-activated cells had easily detectable receptors. TCGF-independent T cells did not bind TCGF. (45 Refs)

16. MARROW COLONY STIMULATING ACTIVITY IN CHRONIC MYELOGENOUS LEUKEMIA.

Bianchi Scarra GL, Barresi R, Sessarego M, Ajmar F, Salvidio E
(c/o Ajmar) Dept. Biology I.S.M.I., Univ. Genova, Viale Benedetto XV, 6, 16132 Genova, Italy
Exp Hematol; 9(9):917-925 1981

Colony stimulating activity (CSA) from bone marrow adherent cells was tested in various myeloproliferative disorders in comparison with chronic myelogenous leukemia (CML). A higher level of medullary CSA was found in acute leukemias with monoblastic component, as contrasted with low values in other acute non lymphocytic leukemias (ANLL). In CML, while the majority of patients had CSA values within normal intervals, few patients had consistently higher levels of this activity. Comparative analysis of growth pattern in agar or in liquid culture suggests that a significant increase in medullary CSA production can be related to an abnormal regulation of granulopoiesis. (Author abstract) (24 Refs)

17. BIOCHEMICAL PROPERTIES OF DIFFERENTIATION FACTORS FOR MURINE MYELOMONOCYTIC LEUKEMIC CELLS IN ORGAN CONDITIONED MEDIA - SEPARATION FROM COLONY-STIMULATING FACTORS.

Nicola NA, Metcalf D
Cancer Res. Unit, Walter and Eliza Hall Inst. Medical Res., P.O. Royal Melbourne Hosp., Parkville, Victoria 3050, Australia
J Cell Physiol; 109(2):253-264 1981

Various murine (C57BL/6f/J/WEHI) organs were surveyed for their capacity to synthesize and release differentiation factor (DF) into the medium. The effect of prior injection of endotoxin (5 ug) on DF production was studied and the biochemical properties of DF produced under various conditions were compared with each other and with the DF found in the serum. Optimal strategy for purifying DF was described, with particular attention given to its separation from the proliferative stimulus colony-stimulating factor (CSF). All organs could induce differentiation of the myelomonocytic leukemic cell lines WEHI-3B, but were much more effective from endotoxin-injected animals. Production of DF was dependent on protein synthesis and occurred throughout the 7-day incubation period. DF produced by the different organs in vitro and found in vivo in endotoxin serum could be distinguished from the majority of granulocyte-macrophage (GM) and macrophage (M) CSF. DF was precipitated by 55% ammonium sulfate and did not bind to Concanavalin A-Sepharose; CSF required 85% saturation and bound to Concanavalin A-Sepharose. DF eluted later than CSF from Phenyl-Sepharose columns. DF could be distinguished from M-CSF by its apparent mol wt on native gels, but could not be distinguished from GM-CSF (23,000 on that basis). DF appears to be a 23,000 (approx) mol wt protein which is different from GM-CSF and may or may not be a glycoprotein. (26 Refs)

18. ROLE OF THE THYMUS IN CFU-S DIFFERENTIATION AND PROLIFERATION.

Lepault F, Fache MP, Frindel E
(c/o Frindel) Laboratoire de Cinetique Cellulaire, Institut Gustave-Roussy, Rue Camille Desmoulins, 94805 Villejuif, France
Leuk Res; 5(4/5):379-383 1981

Data is reported suggesting that the thymus liberates a long-range factor which stimulates erythropoiesis and/or suppresses granulopoiesis at the pluripotent hemopoietic stem cell (CFU-S) level. This factor seems to be different from the factor inducing CFU-S proliferation. It was confirmed that the decision for CFU-S to choose a given pathway(s) of differentiation, ie to become committed, can take place without the CFU-S shifting from resting to active cycling. (26 Refs)

19. SUPPRESSION OF LYMPHOCYTE PROLIFERATION BY HUMAN TUMOR CELLS GROWN IN SHORT TERM CULTURES.

Lafferty WE, Kempf RA, Goodwin JS
(c/o Goodwin) Dept. Medicine, Univ. New Mexico Sch. Medicine, Albuquerque, NM, 87131
J Clin Lab Immunol; 6(2):157-163 1981

The effect of short-term cultures of human tumor cells (less than 10 passages) added to mitogen- and alloantigen-stimulated cultures of peripheral blood monocyte cells (PBMC) on the proliferative responses of PBMC from natural donors was studied. At 1/10 and 1/100 tumor cell/PBMC ratios, only 1/7 tumor cell lines, (a melanoma) caused no suppression of phytohemagglutinin-stimulated cultures. Tumor cells were 50 x more suppressive than normal human fibroblasts. Similar results were obtained using concanavalin A. In mixed lymphocyte cultures, 1/10 tumor cell/lymphocyte ratios resulted in 66-85% suppression, while 1/100 tumor cell/lymphocyte ratios caused 43-66% suppression. Suppression of 3H thymidine incorporation of stimulated lymphocytes by tumor cell line T was evident throughout the 120 hr culture. PBMC recovery and viability were comparable after culture with and without tumor cell T. The percent suppression of 3H thymidine incorporation did not increase as the concentration of 3H thymidine decreased. Glass wool adherent cells were not required for suppression. The inclusion of the prostaglandin-inhibitor indomethacin had no effect on 3H thymidine incorporation by PBMC cultured with tumor cells. Mitomycin C completely abrogated the suppression by tumor T cells while irradiation with 1,000, 2,000, and 3,000 rad had little effect. Tumor cell line T inhibited 3H thymidine incorporation only when incubated in the same chamber with PBMC, not when separated from PBMC by a cell-impermeable membrane. Tumor cell line U, however caused 68% suppression when incubated in the same chamber with PBMC, and 46.4% inhibition across a cell-impermeable membrane. (27 Refs)

20. MACROPHAGES AND RESISTANCE TO TUMORS. THE EFFECTS OF TUMOR CELL PRODUCTS ON MONOCYTOPOIESIS.

Farram E, Nelson M, Nelson DS
Kolling Inst. Medical Res., Royal North Shore Hosp., St. Leonards NSW 2065, Australia
J Reticuloendothel Soc; 30(4):259-269 1981

The formation of macrophage colonies from mouse bone marrow cells was inhibited by supernatants from cultures of cells from certain mouse tumors (a methylcholanthrene-induced and a spontaneous sarcoma) human tumors (a melanoma cell line and HeLa cells) and a normal human cell line (Chang)

but not by supernatants from normal mouse fibroblasts or EL4 mouse lymphoma cells. The active material had an approx mol wt (by ultrafiltration) of less than 1,000. When the active material was injected iv macrophage colony formation of bone marrow cells taken 3 hr later was impaired. Marrow from old and young mice was equally susceptible. One day after tumor isografting, mice showed depressed colony forming capacity accompanied by monocytopenia; this was followed by increased colony forming capacity and monocytosis about 2 wk later. The substance(s) inhibiting macrophage colony formation appears not to be prostaglandin and not the same as that responsible for the depressive effect of tumor supernatants on cellular inflammatory reactions. (Author abstract) (25 Refs)

21. INHIBITION OF GRANULOCYTE-MACROPHAGE (GM) COLONY FORMATION BY BASIC POLYPEPTIDES FROM MOUSE EHRLICH ASCITES TUMOR CELLS.

Szmitkowski M, Gabryel H, Farbiszewski R
Dept. Clinical Diagnostics, Inst. Biochemistry and Medical Analytics, Medical Sch., 15-276 Bialystok, Poland
Experientia; 37(11):1202 1981

The polypeptides isolated from mouse Ehrlich ascites tumor cells were tested for their inhibitory activity against GM colony formation. It was found that basic polypeptides with low mol wt evidently inhibit colony formation. Our data reveal that the tested polypeptides may show chalone activity. (Author abstract) (12 Refs)

22. INHIBITORY EFFECT OF HUMAN URINARY EXTRACT ON THE GROWTH OF DMBA-INDUCED MAMMARY TUMORS IN HOLTZMAN RAT.

Sarkar S, Shah PN, Bagli NP
Cancer Res. Inst., Bombay 400 012, India
Neoplasma; 28(4):457-461 1981

The effect of a crude protein fraction from human urine having gonadotropin inhibitory activity (CGIM) was observed on the growth of 7,12-dimethylbenz(a)anthracene induced mammary tumors in the Holtzman rat. Results show that while in 12 out of 13 tumors, tumor growth was retarded/regressed following 2-4 wk of CGIM treatment, tumor growth in control rats was unabated. However, no positive correlation with hormone dependency and tumor regression could be established. Further, this crude anti LH material was found to be non-toxic to tumor cells in vitro. (Author abstract) (11 Refs)

23. EFFECT OF EPIDERMAL CHALONES ON CELL PROLIFERATION AND DIFFERENTIATION IN MURINE TUMOR OF THE LOWER LIP.

Godorozha PD, Zabezhinskii MA, Okulov VB, Puchkov IuG, Pliss GB
N. N. Petrov Res. Inst. Oncology, Leningrad, USSR
Biull Eksp Biol Med; 91(9):347-349 1981

The effect of epidermal chalones (EC) on tumor progression was studied in CC57BR mice bearing methylcholanthrene (MC)-induced lower lip tumors. Simultaneous application of MC and EC prolonged the lifespan of the tumor-bearing animals (192 days, compared with 164 days for mice treated with MC alone and 141 days for mice treated with MC and liver chalone). Application of EC after te rmination of application of MC also inhibited tumor growth (av lifespan, 195 days). The application of the EC enhanced differentiation of the tumor cells and reduced the depth of invasion. (10 Refs) (In RUS)

HORMONES/ STEROIDS/ PROSTAGLANDINS AS GROWTH EFFECTORS

24. CORRELATION OF CIRCULATING LEVELS OF A SERUM PROTEIN WITH TRIIODOTHYRONINE LEVELS AND HEPATOMA GROWTH.

Coetzee ML, Short J, Klein K, Ove P
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Cancer Res; 42(1):155-160 1982

In this communication, we provide evidence that proliferation of transplantable Morris hepatoma 7777 might to some extent be regulated by triiodothyronine and/or a specific serum protein, the levels of which are correlated with levels of triiodothyronine. The protein has an estimated mol wt of 80,000 and migrates as one band on polyacrylamide gel electrophoresis. In normal rats, this protein accounts for approx 1% of the total serum protein. Both the circulating levels of the serum protein and proliferation of transplanted hepatoma cells were decreased in thyroidectomized rats. Elevated levels of the serum protein and increased cell proliferation were observed when animals had 70% of their liver removed prior to transplant, were given injections of triiodothyronine, or had a 10-day-old first transplant surgically removed. Some evidence is also provided suggesting that the synthesis of the serum protein is stimulated by thyroid hormone. (Author abstract) (36 Refs)

25. EFFECTS OF INSULIN ON CELLULAR GROWTH AND PROLIFERATION.

Straus DS
Biomedical Sciences Div., Univ. California, Riverside, CA, 92521
Life Sci; 29(21):2131-2139 1981

Insulin stimulates the growth and proliferation of a variety of cells in culture. The growth-stimulatory effects of insulin are observed in G0/G1 arrested cells limited for serum growth factors or essential nutrients, and in cells growing in hormone-supplemented serum-free media. Some, but not all, of the effects of insulin on growth require superphysiological concentrations of insulin. The action of insulin on growth is synergistic with the action of other hormones and growth factors, including FGF, PDGF, PGF2alpha, and vasopressin. This observation, as well as other observations regarding the temporal sequence of action of growth factors, suggests that different growth factors act on different intracellular biochemical events. Several hypotheses have been proposed to explain the effect of insulin on cellular proliferation, including regulation of essential metabolic processes and interaction of insulin with receptors for insulin-like growth factors. Evidence supporting these various hypotheses is reviewed. In addition to the growth-stimulatory effect of insulin observed in cell culture, a number of clinical examples sugges t that insulin is an important growth-regulating hormone during fetal development. (Author abstract) (87 Refs)

26. THE ROLE OF ERYTHROPOIETIN IN ERYTHROID COLONY FORMATION FROM RAUSCHER LEUKEMIA VIRUS-INFECTED MICE.

Gallicchio VS, Murphy MJ
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Exp Hematol; 9(9):938-944 1981

Recent work has led to conflicting results regarding the in vitro control of erythropoiesis in mice infected with Rauscher Leukemia Virus (RLV). Some reports claimed that marrow or splenic erythropoiesis from RLV-infected mice were independent of exogenously added erythropoietin (Ep), while other reports supported the view that erythroid stem cells (CFUE) from RLV-infected mice required Ep for erythroid differentiation and proliferation. Results reported here demonstrate that CFUE cultured from RLV-inoculated mice are dependent upon Ep for erythroid differentiation. Furthermore, RLV-infected mice which had received transfusions to suppress the endogenous production of Ep not only had a reduction in spleen wt and reticulocyte percentage, but also reduced numbers of CFUE and BFUE. (Author abstract) (20 Refs)

27. MODULATION OF HUMAN MELANOMA COLONY FORMATION IN SOFT AGAR BY LUTEINIZING HORMONE (MEETING ABSTRACT).

Meyskens FL, Sander D, Kramb P
Department of Internal Medicine and Cancer Center, University of Arizona, Tucson, AZ
Third European Workshop on Melanin Pigmentation, September 28-October 1, 1981, Prague, Czechoslovakia. Czechoslovak Medical Society 128 pp., 1981.

Clinical and experimental data with animal and human models suggest that gonadotropins regulate melanoma growth. Cells were obtained from biopsy of 5 patients with metastatic melanoma. The effect of continuous contact of human luteinizing hormone ((hLH) 0.01 to 100 ug/ml) on melanoma colony formation in soft agar was assessed by visual observation and enumerated by FAS II optical scanning. Two patterns of response to hLH were demonstrated. In two patients (80-62) 80-63) hLH stimulated clonogenic cells of different proliferative capacities (ie colonies greater than 60, greater than 86, greater than 104u diameter) to a similar degree. In 3 patients (80-54, 81-3, 81-6) the stimulating effect was greater on clonogenic cells with more proliferative capacity. Since clonogenic cells with the most proliferative capacity (ie larger colonies) represent only a small proportion of the clonogenic cell compartment (eg in patient 81-6, colonies greater than 104u diameter in control cultures represent only 6% of the total clonogenic cell population (4/61, greater than 60u)); hLH may have its major modulating role in melanoma on an early melanoma cell progenitor in these patients. (no Refs)

28. PANCREATIC CANCER GROWTH STIMULATION BY CAERULEIN AND SECRETIN (MEETING ABSTRACT).

Townsend CM, Franklin RB, Watson LC, Glass EJ, Thompson JC
Department of Surgery, University of Texas Medical Branch, Galveston, TX, 77550
Newsletter Natl Pancreas Cancer Project; VI(2):59 1981

Caerulein (C), alone, or in combination with secretin (S) produces hyperplasia of the normal rat pancreas. The effects of these hormones on pancreatic ductal cancer (as well as normal hamster pancreas) are unknown. The purpose of this study was to determine whether pancreatic trophic hormones stimulate growth of pancreatic ductal cancer in Syrian golden hamsters. We used a tissue culture cell line of hamster pancreatic ductal cancer developed in our laboratory. Groups of 8 male hamsters were inoculated with 5x10(5) cells into the left cheek pouches. After 7 days of tumor growth, treatment was begun with 0.15 M NaCl for controls (CON), and with either C (15 ug/kg/day) or S (75 ug/kg/day), or a combination of C + S. Divided daily doses were given by ip injection (0.5 ml) every 8 hr for 15 or 20 days. At sacrifice, tumors (T) and normal pancreas (P) were excised, weighed, and the DNA content determined. Trophic hormones for normal hamster pancreas appear to stimulate growth of pancreatic cancer. To our knowledge, this is the first demonstrated endocrine stimulation of pancreatic ductal carcinoma. (no Refs)

29. EFFECTS OF 17BETA-ESTRADIOL ON PROGESTERONE RECEPTORS AND THE UPTAKE OF THYMIDINE IN HUMAN BREAST CANCER CELL LINE CAMA-1.

Yu WC, Leung BS, Gao YL
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Cancer Res; 41(12):5004-5009 1981

Two sublines, CAMA-1R and CAMA-1N, were derived from the original CAMA-1 cells after continuous passages in our laboratory. 1R cells were supplemented in culture with estradiol and retained the properties of the original cell line, whereas 1N cells, not supplemented with estradiol, had a partial loss of estrogenic responsiveness. The doubling times for 1R and 1N cells were 2 and 2.4, respectively. These two sublines showed different effects on thymidine uptake after estradiol stimulation. Cells of 1R had a 2-fold greater thymidine incorporation than did the control without estradiol. In contrast, no difference of thymidine uptake was demonstrated in cells of 1N in the presence or absence of estradiol. Both cell lines contained cytoplasmic progesterone receptors with a Kd of 0.56 nM and a sedimentation coefficient of 6 to 7S on a 5 to 20% sucrose gradient. The progesterone receptor level in 1N was about 8-fold higher than in 1R cells. Both sublines exhibited a dose-dependent increase in progesterone receptor levels in response to estradiol with the max stimulation (8-fold increase) reached by using 5 nmol of estradiol. The rate of increase for estradiol-induced progesterone receptor appears to be the same for both sublines ; a linear increase was demonstrated between Days 3 and 7 following plating. Our results show that estrogen regulation of thymidine uptake and progesterone receptor induction may be mediated through two separate mechanisms. Thus, following a chronically deficient supply of estradiol to 1N cells, there is apparent loss of responsiveness to estradiol-induced thymidine uptake, but the ability of these cells to respond to estradiol induction of progesterone receptor was retained. (Author abstract) (30 Refs)

30. EFFECT OF ESTROGEN AND PROGESTERONE ON CELLULAR REPLICATION OF HUMAN BREAST TUMORS.

Dao TL, Sinha DK, Nemoto T, Patel J
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Cancer Res; 42(1):359-362 1982

A total of 54 infiltrating carcinomas of the breast were studied for estrogen receptor concentration in the tumor cytosol and thymidine-labeling indexes. The results showed that there was no significant difference in the level of thymidine-labeling index between 22 primary and 32 metastatic breast cancers. No significant association between the levels of thymidine-labeling

index and the presence or absence of estrogen receptors was observed. The effect of 'physiological' doses of estrogen and progesterone on cell proliferative activity was studied by the level of thymidine-labeling indexes in the tumor cells in 10 patients with multiple skin and sc metastases. Tumor biopsies were performed for labeling indexes both before and after hormonal treatment. The results showed that physiological doses of estrogen and progesterone induced a significant rise in thymidine-labeling index within 3 days after hormonal treatment in 7 of the 10 tumors. Of the seven tumors that showed a rise in thymidine-labeling index, three were estrogen receptor positive and four were estrogen receptor negative. Of the three nonresponsive tumors, one was estrogen receptor positive and two were estrogen receptor negative. The study suggests that estrogen and progesterone can induce cell replication in both estrogen receptor-positive and -negative tumors. (Author abstract) (18 Refs)

31. TAMOXIFEN AND METABOLITES IN MCF7 CELLS: CORRELATION BETWEEN BINDING TO ESTROGEN RECEPTOR AND INHIBITION OF CELL GROWTH.

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Cancer Res; 42(1):317-323 1982

The binding of [3H]tamoxifen ([3H]Tam), a nonsteroidal antiestrogen, and of 4-[3H]hydroxytamoxifen ([3H]OH-Tam), a metabolite accumulated in vivo in target cell nuclei, was characterized in soluble extracts of human breast cancer MCF7 cells growing in a medium depleted in estrogens. Saturation analysis indicated a much higher affinity of OH-Tam (Kd = 0.15 nM) than for Tam (Kd = 4.8 nM). The bi nding of [3H]Tam and [3H]estradiol was competitive and mutually exclusive, and the binding site concentration (0.16 to 0.47 pmol/mg total protein) was similar for both ligands, strongly suggesting that antiestrogens were binding to the estrogen receptor (ER) in these cells. The ability of Tam and of some of its metabolites or derivatives to prevent the MCF7 cell growth was found to be correlated with their affinity for ER as determined by direct interaction or by binding competition with [3H]estradiol on the uterine and MCF7 cytosol ER. OH-Tam was the highest-affinity compound and was 100-fold more active than Tam. The inhibitions observed were actually due to Tam and OH-Tam, respectively, since we did not detect any significant metabolism of these two labeled compounds by the MCF7 cells. N-desmethyltamoxifen, the other Tam metabolite found in high concentration in human plasma, was as effective as Tam while cis-tamoxifen appeared less effective. Compound E, which has no lateral chain, was the only tested compound having a good affinity for ER and a poor efficiency in preventing cell growth. These results support the hypothesis that antiestrogens control the growth of breast cancer by acting directly on the ER located in cancer cells. (Author abstract) (33 Refs)

32. THROMBOXANES AND PROSTACYCLIN: POSITIVE AND NEGATIVE MODULATORS OF TUMOR GROWTH.

Honn KV, Meyer J
Dept. Radiation Oncology, Wayne State Univ., Detroit, MI, 48202
Biochem Biophys Res Commun; 102(4):1122-1129 1981

The effects of thromboxane A2 (TXA2), thromboxane synthetase inhibitors, and prostacyclin (PG12) on the proliferation of B16 amelanotic melanoma cells were examined. Both TXA2 and the TXA2-mimicking agent TXB2 [(15S)-hydroxy-11alpha,9alpha-(epoxymethano)prosta-5Z, 13E-dienoic acid] significantly stimulated tumor cell proliferation in a dose-dependent manner. Incubation of B16a cells with TXB2 (25 ug/ml) for 4 hr decreased the basal level of intracellular cyclic AMP and cyclic AMP released into the media without affecting cyclic GMP levels; preincubation of the cells with TXB2 (25 ug/ml) for 48 hr inhibited the increase in intracellular cyclic AMP release normally seen in response to incubation with prostaglandin E1 (PGE1: 1 ug/ml). Treatment of cultured B16a melanoma cells with either PG12 or with thromboxane synthetase inhibitors produced a significant dose-related inhibition of proliferation at noncytotoxic doses. Thromboxane synthetase inhibitors and a TXA2 receptor antagonist significantly reduced DNA synthesis by B16a cells at noncytotoxic doses. The results suggest that ther is bidirectional control of tumor cell proliferation by the balance between endogenous and/or exogenous PG12/TXA2 and that this regulation of tumor cell proliferation may center on the modulation of tumor cell cyclic AMP levels. The modulation of PG12/TXA2 ratios m ay be an important therapeutic locus in the control of tumor growth. (26 Refs)

33. INHIBITION OF EPIDERMAL GROWTH FACTOR BINDING BY PROSTAGLANDIN A'S.

Lee LS
Dept. Res. Development, General Electric, Schenectady, NY
Biochem Biophys Res Commun; 102(4):1144-1150 1981

The effects of various prostaglandins on the binding of 125I-labeled epidermal growth factor (EGF) to a number of cell lines were investigated. Prostaglandin A1 and prostaglandin A2 (16 ug/ml) exerted rapid, dose-related inhibition of 125I-EGF binding to cell lines MRC-5, HeLa, XP, WI38, and 10T1/2, whereas prostaglandins E1, E2, F1-alpha, B2 and F2-alpha-tromathamine in the concentration range 0.026-54 ug/ml had little or no effect. The 50% binding-inhibition doses for PGA1 and PGA2 in HeLa cells were 7 ug/ml for 17 ug/ml, respectively, when tested with 125I-EGF at 0.5 nanog/10(6) cells. PGA1 and PGA2 did not inhibit the binding of labeled insulin, multiplication-stimulating activity, human growth hormone, concanavalin A, or phorbol 12,13-dibutyrate. These results show that the A-series prostaglandins can be used as a probe to investigate the EGF-receptor system. Since compounds that inhibit EGF binding are often tumor promoters, the results also suggest that substances such as PGA's, when present at high concentrations, may be tumor promoting. (25 Refs)

34. PROSTAGLANDINS AND ERYTHROPOIESIS: STRUCTURE/ACTION RELATIONSHIPS AND IDENTIFICATION OF THE PROSTAGLANDIN RESPONSIVE CELLS.

Dunn CD
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Blut; 42(5):307-314 1981

The effects of prostaglandins (PG) on erythropoiesis were studied in serum-free cultures of fetal mouse liver cells (FMLC). PG derivatives studied included PGE2, PGE1, PGA2, PGA1, PGB2, PGB1, PGF2alpha (PGF2a), 6-keto PGF1alpha (6-kPGFa), 15 epi PGF2alpha (15-ePGF), and prostacylcin. Molar concentrations necessary to produce 50% erythropoietin (Ep)-controlled heme synthesis in FMLC were PGE2 5 x 10(-15), PGF2a 10(-15), and PGB1 10(-9). Inhibition of heme synthesis was produced by seven PG derivatives at concentrations ranging from 5 x 10(-11) to 10(-5) M. No effects were produced by 15-ePGF, PGB2, or prostacyclin. The effect

of PGE2 was inhibited by 15-ePGF, but the effect of Ep was not. Sedimentation studies indicated that PGE2 was active on a subpopulation of larger cells within the Ep-responsive cell population. (17 Refs)

35. PROSTAGLANDINS AND CANCER: A REVIEW OF TUMOR INITIATION THROUGH TUMOR METASTASIS.

Honn KV, Bockman RS, Marnett LJ
Dept. Biological Sciences, Wayne State Univ., Detroit, MI, 48202
Prostaglandins; 21(5):833-864 1981

The role of prostaglandins (PG) in tumor initiation, promotion, and metastasis is reviewed. Results from tests in Salmonella typhimurium and mice suggest that prostaglandin endoperoxide synthetase (PES) may play a role in activating carcinogenic compounds, such as malondialdehyde, although findings may have been affected by carcinogenic impurities. Arachidonic acid may trigger cooxidation of noncarcinogens, such as benzo[a]pyrene to carcinogenic forms. The kidney may play a role in toxicity or carcinogenicity because of its physiological composition and high blood flow. PES appears to trigger carcinogens when introduced into dog kidneys in vivo. PG may play a role in tumor promotion by tetradecanoyl phorbol acetate (TPA). TPA treatments result in increased PG levels. Nontumorigenic phorbol esters do not increase PG levels. Some inhibitors of PES and lipoxygenases have been found to inhibit tumor growth in mice. PG apparently play a role in proliferation and differentiation of cells and therefore may affect tumor growth. PG appear to inhibit proliferation of monocyte and T lymphocyte precursors and to enhance proliferation of erythrocytes. Manipulation of PG levels may offer a means of controlling tumor growth and metastasis. (161 Refs)

CYCLIC NUCLEOTIDE/ POLYAMINES/ NUCLEOTIDES AS GROWTH EFFECTORS

36. CHANGES IN CYCLIC NUCLEOTIDE LEVELS DURING ERYTHROID DIFFERENTIATION IN FRIEND LEUKEMIA CELLS.

Chapman LF
Div. Biological Sciences, Univ. Missouri, Columbia, MO, 65211
Exp Cell Res; 135(2):415-418 1981

Changes in cyclic nucleotide levels were observed in Friend leukemia cells exposed to the inducers of erythroid differentiation dimethylsulfoxide (DMSO), sodium butyrate and hypoxanthine . The most extensive changes in cAMP levels occurred during the initial 24 hr growth period. In uninduced cultures, cAMP concentration increased 35-fold compared with a 7-fold increase in the presence of inducer. During the same period, cGMP levels rose about 3-fold in uninduced cells compared with a 20-fold increase in induced cells. (Author abstract) (11 Refs)

37. CYCLIC NUCLEOTIDES AND CALCIUM IN HUMAN LYMPHOCYTES INDUCED TO DIVIDE.

Rochette-Egly C, Kempf J
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J Physiol (Paris); 77(6/7):721-725 1981

Intracellular concentrations of cyclic AMP (cAMP), and cyclic guanosine monophosphate (cGMP) were measured in lymphocytes triggered by concanavalin A, phytohemagglutinin, 12-O-tetradecanoylphorbol-13-acetate and the calcium ionophore A 23187. cGMP rose within min without concomitant cAMP alteration. The cAMP and cGMP levels rose during the prereplicative and replicative phases, respectively. Under calcium depleting conditions, the fluctuations in cAMP and cGMP and increase in (3H)thymidine incorporation into DNA were abolished, suggesting a role for Ca++ in the regulation of lymphocyte proliferation. (21 Refs)

COFACTORS/ NUTRITIONAL GROWTH FACTORS

38. GROWTH STIMULATION OF BOVINE ENDOTHELIAL CELLS BY VITAMIN A.

Melnykovych G, Clowes KK
Medical Res. Service, Veterans Admin. Medical Center, Kansas City, MO, 64128
J Cell Physiol; 109(2):265-270 1981

The effect on growth of bovine aortic endothelial cells maintained in minimal essential medium (MEM) supplemented with delipidized serum by retinol at concentrations of 10(-8) to 10(-5) M was investigated. Without retinol, the increase in cell number was minimal, although there was no cell loss. In retinol-treated cultures, the increase in cell number, although modest in comparison with cells grown in complete medium, was more than four-fold. Some slight stimulation was seen at 10(-8) and 10(-7) M retinol, and significant stimulation was seen at 10(-6) and 10(-5) M. Above 10(-5) M retinol was toxic causing cell detachment from the substratum and eventually cell disintegration. When the effects of other members of the vitamin A group were investigated, similar stimulation in cell growth was seen after 3 days of culture. It was difficult to achieve growth of bovine endothelial cells in media containing delipidized serum starting from inocula at low densities commonly used for cloning cells. However, a growth stimulatory effect of retinol under low inoculum conditions was demonstrated by the use of conditioned medium from P388D1 cultures (derived from mouse lymphoma). At inoculations of 4-40 cells/square cm, the growth promoting effect of retinol could only be demonstrated in the presence of conditioned medium from macrophage-like culture P388D1. Retinol was growth inhibitory when added to media containing whole (not delipidized) serum at concentrations of 10(-6) and 10(-5) M. In the absence of retinol, P388D1-conditioned medium did not by itself stimulate formation of endothelial cell colonies. (20 Refs)

39. REGULATION OF PROLIFERATION OF NORMAL AND LEUKEMIC MYELOID CELLS BY RETINOIC ACID IN VITRO (MEETING ABSTRACT).

Doner D, Koeffler HP
Department of Medicine, University of California of Los Angeles School of Medicine, Los Angeles, CA, 90024
Exp Hematol; 9(Suppl9):195 1981

We have found that all-trans and 13-cis retinoic acid at 3 x 10-7M increased by about 2-fold the number of normal human granulocyte-macrophage colony forming units (CFU-GM) formed in soft agar culture in the presence of colony stimulating factor (CSF). Retinal, retinyl acetate and the trimethylmethoxphenyl ethyl ester analog of retinoic acid (RIO-9359) were less potent than retinoic acid, while retinol (vitamin A) had no effect on colony formation. We tested the

ability of retinoic acid to enhance CFU-GM formation in the presence of a variety of sources of CSF such as conditioned medium (CM) from a human T-lymphocyte cell line (Mo line), CM from stimulated mononuclear cells, and human peripheral blood white cell feeder layers. With each of these sources of CSF, 3 x 10(-7) retinoic acid increased the number of CFU-GM by 1.7-t o 2-fold as compared to plates containing the CSF alone. The effect of retinoic acid on the clonal growth of myeloid leukemia cells was studied by culturing in soft agar cells from three human myeloid leukemia lines (KG-1, HL-60, and K562), and cells from a mouse myeloid leukemia line (M-1). About 50% of the colonies were inhibited at a retinoic acid concentration of 10-9M for KG-1 and 10-8M for HL-60; and no KG-1 or HL-60 colonies formed when plated with 10-5M retinoic acid. In contrast, retinoic acid had no effect on the number of colonies formed by the K562 and M-1 cell lines. Our results show that retinoic acid enhances normal myeloid colony formation and inhibits certain myeloid leukemia clonal growth in vitro. These opposing in vitro effects of retinoic acid may be therapeutically useful in certain myeloid leukemia patients. (no Refs)

EXOGENOUS AGENTS AFFECTING DIFFERENTIATION

40. GLUCOCORTICOID-STIMULATED INCREASE IN CHEMOTACTIC PEPTIDE RECEPTORS ON DIFFERENTIATING HUMAN MYELOID LEUKEMIA (HL-60) CELLS.

Brandt SJ, Barnes KC, Glass DB, Kinkade JM
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Cancer Res; 41(12):4947-4951 1981

The effect of dexamethasone on the HL-60 human promyelocytic leukemia cell line was studied using a radioligand-binding assay for a chemotactic peptide receptor that is found on the surface of mature polymorphonuclear leukocytes. When dimethyl sulfoxide (DMSO) is added to cultures, HL-60 cells undergo morphological and functional differentiation over a period of 6 to 7 days. Differentiation is accompanied by increased binding of the synthetic N-formylated chemotactic peptide N-formylmethionylleucyl[3H]phenylalanine. Binding of N-formylmethionylleucyl[3H]phenylalanine to cells induced to differentiate with DMSO was temperature dependent, specific, saturable, of high affinity, reversible, and proportional to cell number. Dexamethasone increased the number of N-formylated chemotactic peptide receptors in cultures of differentiating HL-60 cells without affecting the affinity of the receptor for the tritiated peptide. This steroid response was dose dependent, proportional in magnitude to glucocorticoid activity, and abolished by cycloheximide. Dexamethasone had little effect on N-formylmethionylleucyl[3H]phenylalanine binding unless DMSO was also present in culture or the cells were first induced by DMSO to differentiate. These results suggest that the glucocorticoid stimulated increase in number of N-formylated chemotactic peptide receptors was mediated by the high-affinity glucocorticoid receptor, involved protein synthesis, and was apparently dependent on differentiation. (Author abstract) (33 Refs)

41. MECHANISMS FOR INDUCTION OF DIFFERENTIATION IN HUMAN MYELOID LEUKEMIC CELL LINES (HL-60 AND U-937) (MEETING ABSTRACT).

Olsson I, Breitman TR, Keene BR
Department of Medicine, University of Lund, S-221 85 Lund, Sweden
Exp Hematol; 9(Suppl9):41 1981

In myeloid leukemia differentiation seems to be blocked at various stages of maturation. The HL-60 cell line consists predominantly of promyelocytes and is induced to differentiate to morphologically and functionally mature granulocytes by incubation with a wide variety of compounds including dimethyl sulfoxide (DMSO) and retinoic acid (RA). U-937 is a malignant monoblastic cell line. RA but not DMSO was found to induce differentiation of U-937 into monocyte like cells with the capacity to reduce nitroblue tetrazolium (NBT). In addition, mitogen stimulated mononuclear blood cells produce differentiation inducing factors (DIFs) for HL-60. Such factors are produced constitutively by some malignant T-lymphocyte lines. An activity, produced by mitogen-stimulated normal mononuclear blood cells or unstimulated malignant T-lymphocytes, cochromatographed with DIF and acts synergistically with RA to induce maturation of HL-60 and U-937. Thus the combination of 10 nM RA and DIF induced the maturation of 70-80% of HL-60 cells. The combination of 100 nM RA and DIF induced the maturation of 50% of U-937 cells. Furthermore, compounds elevating the intracellular level of cyclicAMP (cAMP) (eg dibuturyl cAMP, prostaglandins and choleratoxin) acted synergistically with RA to induce maturation of both cell lines. This maturation is, however, not dependent on the continued presence of RA as DIFs and cAMP agents are active also on cells primed for 8-16 hr with 10 nM RA. Cells primed with 10 nM RA also matured in response to exogenous ATP. There was an increase in the extent of phosphorylation of cell surface protein(s) as measured by 32P incorporation from [gamma-32P]-ATP with intact HL-60 cells previously treated for 8-16 hr with RA. (no Refs)

42. FATTY ACID METABOLISM IN PHORBOL ESTER-DIFFERENTIATING HUMAN LEUKEMIA CELLS.

Cabot MC, Welsh CJ
Medical Health Sciences Div., Oak Ridge Associated Univ., Oak Ridge, TN, 37830
Cancer Res; 41(12):4910-4915 1981

Human promyelocytic leukemia cells (HL-60) undergo differentiation when treated with the tumor promoter, 12-O-tetradecanoylphorbol-13-acetate (TPA). As the primary target for TPA action is membranes, studies were undertaken to determine whether phorbol ester exposure would influence fatty acid metabolism in these cells. In cells incubated with labeled fatty acids for 1 hr, the percentage of distribution of lipid radioactivity is highest in the phospholipid fraction of control cultures, whereas in TPA-supplemented cells, substantially more label is associated with triacylglycerols. The specific activity of phospholipids and triacylglycerols was lower in treated than in control cells; however, the amount of cellular triacylglycerols increased 3.2-fold (lipid per mg protein). The increase in the amount of cellular phospholipids in TPA-treated cells is not as pronounced (approx 50% above control), and this only occurs at higher concentrations of TPA. At early times after TPA exposure, there is no stimulation of the cellular uptake of labeled fatty acids; however, differentiating cells (24 to 48 hr of TPA), when incubated with label, contained more radioactivity than did control cultures. Cells treated with TPA for 48 hr show a marked decrease in the conversion of [1-14C]stearic acid to monoenoic product (22% of control);

this decrease is dose dependent and occurs within 24 to 48 hr of treatment. Although the phospholipid fatty acid composition of differentiating cells was similar to control cells, acyl groups of triacylglycerols isolated from treated cells showed a marked decrease in the percentage of unsaturates. These data provide evidence which demonstrates that TPA treatment of HL-60 cells has a profound effect on fatty acid metabolism. The lack of an effect of TPA on fatty acid metabolism after short-term exposure to the promoter suggests that the modifications observed may be the result of cellular differentiation rather than a direct effect exerted by the presence of TPA in the culture media. (Author abstract) (26 Refs)

43. INDUCTION OF HEMOGLOBIN SYNTHESIS IN ORIGINAL K 562 CELL LINE.

Fuhr JE, Bamberg EG, Lozzio CB, Lozzio BB

Dept. Medical Biology, Univ. Tennessee/Memorial Res. Center, Knoxville, TN, 37920

Blood Cells; 7(2):389-395 1981

Cells of the original human myelogenous leukemia cell line, K 562, were induced to synthesize Hb by incubation with 0.05 milliM hemin. Isoelectric focusing indicated major bands in the Hb F region, Bart's Hb, and embryonics. Cytochemical analysis by Lepehene peroxidase reaction for Hb indicated an heterogeneous population of cells with respect to Hb induction. Benzidine-positive material in the form of granules were found in the region of the Golgi apparatus of large blasts with undifferentiated nuclei. There was no indication of normal maturation or of terminal differentiation into reticulocytes or RBC. (10 Refs)

44. DEXAMETHASONE INHIBITS A HEME-INDEPENDENT EVENT NECESSARY FOR TERMINAL DIFFERENTIATION OF MURINE ERYTHROLEUKEMIA CELLS.

Hugues B, Osborne HB

Laboratoire de Biologie Moleculaire et Cellulaire, E.R. 199 du C.N.R.S., Dept. de Recherche Fondamentale Centre d'Etudes Nucleaires de Grenoble, 85X, 38041, Grenoble cedex, France

Biochem Biophys Res Commun; 102(4):1342-1349 1981

The effect of exogeneous hemin on the inhibition, by dexamethasone (DEX), of hexamethylene bisacetamide (HMBA)-induced differentiation of murine erythroleukemia cells was investigated. When 100 uM hemin was added to cells cultured for 44 hr in the presence of 4 milliM HMBA and 100 nanoM DEX, there was an increase in the proportion of benzidine-positive cells, which was due to the appearance of Hb in the cytoplasm of these cells. Cells cultured in the presence of HMBA were shown to have a low plating efficiency due to their commitment to terminal differentiation. However, although the addition of hemin to the culture with HMBA and DEX caused the proportion of benzidine-positive cells to rise to more than 80%, the plating efficiency was not reduced. This indicate that the addition of hemin did not override the inhibition by DEX of commitment to terminal differentiation whereas the inhibition of the appearance of Hb was suppressed. The induction of the nuclear protein IP25, which is associated with reprogramming to limited growth ability in several other systems, was not inhibited by DEX. These experiments show that DEX inhibits at least one heme-independent step necessary for commitment to terminal differentiation but not for globin gene expression. (25 Refs)

45. CHANGES IN SURFACE ARCHITECTURE DURING MURINE ERYTHROLEUKEMIA CELL DIFFERENTIATION AS DETECTED BY LECTIN BINDING AND AGGLUTINATION.

Tsiftsoglou AS, Sartorelli AC

Dept. Medicine, Hematology-Oncology Unit, Harvard Medical Sch., Beth Israel Hosp., 330 Brookline Ave., Boston, MA, 02115

Biochim Biophys Acta; 649(1):105-112 1981

Cell surface alterations occurred during murine erythroleukemia cell (clone 745) differentiation that were detected by both agglutination and lectin binding. Agglutination of erythroleukemia cells were produced by wheat germ agglutinin; whereas, concanavalin A, ricin, soybean agglutinin and fucose-binding protein were either ineffective or much less efficacious. Treatment of leukemia cells with the inducer of erythroid differentiation dimethylsulfoxide (DMSO) caused a progressive accumulation of Hb-containing cells in culture and a decrease in the rate of agglutination by wheat germ agglutinin, which began at 24 hr after exposure to the polar solvent, reached a nadir at 48 hr, and remained essentially constant thereafter. The binding of radioactive wheat germ agglutinin by untreated control erythroleukemia cells increased with time in culture, reaching a max value at 48 hr, and decreased progressively thereafter. Although an increase in 3H-labeled wheat germ agglutinin binding also occurred in DMSO-treated cells, the level bound was significantly lower than that observed in control cells at 24-96 hr. The treatment of erythroleukemia cells with various concentrations of DMSO resulted in a decrease in the number of wheat germ agglutinin receptor sites. Other inducers of differentiation (ie, dimethylformamide, bis(acetyl)diaminopentane) also inhibited the rate of wheat germ agglutinin-induced agglutination of erythroleukemia cells while, in contrast, the inducer tetramethylurea did not. These studies indicate that membrane changes occur duri ng differentiation and suggest that there may be more than one mechanism involved in the initiation of maturation which ultimately leads to the common pathway of erythroid development. (Author abstract) (39 Refs)

46. CFU-S DIFFERENTIATION AFTER MULTIPLE DOSES OF CYTOSINE ARABINOSIDE: EFFECT OF INHIBITORS.

Guigon M, Dumenil D, Enoug J, Frindel E

(c/o Frindel) Laboratoire de Cinetique Cellulaire, Institut Gustave-Roussy, Rue Camille Desmoulins, 94805 Villejuif, France

Leuk Res; 5(4/5):367-372 1981

The ratio of erythroid and granulocytic colonies in the recipient spleen as assessed by histological examination (EG ratio) generated by pluripotent stem cells of the hematopoietic tissues (CFU-S) was determined after multiple doses of cytarabine (Ara-C): 1 injection of 5 mg, 3 injections of 10 mg at 8-intervals, and 5 injections of 5 mg at 24-hr intervals. The E/G ratios, which were 2.1 for normal bone marrow, increased significantly after a single dose of Ara-C, whatever the dose. These high values (4.3-5.2) resulted from an increase in the number of erythroid colonies and a decrease in the number of granulocytic colonies. When 3 10-mg injections were given, the E/G ratio decreased to 1.1, 8 hr after the third injection. After 5 injections of 5 mg, the E/G ratio was dramatically decreased (0.4-0.6) due to a decreased number of erythroid colonies with an increase of granulocytic colonies, along with a considerable increase in the number of megakaryocytic colonies with slight modifications of the undifferentiated colony numbers. With Ara-C alone, the E/G ratio was decreased (1.1) compared to control untreated mice (2.1); with the simultaneous administration of an CFU-S inhibitor either as a crude

dialysate or a purified fraction, the E/G ratio was increased (3.2-6.8) above the normal value and reached values observed after one 20-mg injection of Ara-C. In those experiments in which the E/G ratio was increased, due to injection of inhibitors, an increase in the survival of CFU-S was observed. When the dialysate did not increase CFU-S survival, it also had no effect on the E/G ratio. Assuming that E/G ratios may reflect the modifications of CFU-S cell lineage determination, it was suggested that various insults to the hematopoietic tissue trigger a cascade of events that are responsible for the modifications of CFU-S differentiation pathways. (23 Refs)

47. THE EFFECT OF DIVALENT CATIONS ON THE PROLIFERATION AND DIFFERENTIATION OF CLOUDMAN S 91 MELANOMA CELL LINE (MEETING ABSTRACT).

Borovansky J, Riley PA
Department of Biochemical Pathology, University College School of Medicine, London WC1E 6JJ, England
Third European Workshop on Melanin Pigmentation, September 28-October 1, 1981, Prague, Czechoslovakia. Czechoslovak Medical Society 128 pp., 1981.

The effect of Ca++, Cd++, Cu++, Mg++ and Zn++ as acetates on the proliferation a nd differentiation of clone M3 of Cloudman mouse melanoma was studied and compared with the behavior of GPK (keratocyte) and MRC5 (fibroblast) cell lines. Whereas neither calcium nor magnesium ions influenced the proliferation of the cells as measured by 3H-thymidine incorporation, cell counts and spectral extinction at 280nm of NaOH cell digests, zinc, cadmium and copper ions selectively inhibited the melanoma line. Cd++ (10 uM) and Zn++ (100 uM) were cytotoxic to melanoma cells but stimulated growth by keratocytes and fibroblasts. No direct effect on melanogenesis, as estimated from the ratio of spectral extinction at 350nm and 280nm and by tyrosinase assays, was demonstrated. Experiments with ouabain indicate that active transport is involved in the uptake of zinc by melanoma cells. (no Refs)

OTHER RELATED STUDIES

48. CHARACTERIZATION OF THE PHYTOHEMAGGLUTININ-INDUCED PROLIFERATING LYMPHOCYTE SUBPOPULATIONS IN CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS USING A CLONOGENIC AGAR TECHNIQUE AND MONOCLONAL ANTIBODIES.

Davis S
Dept. Oncology/Hematology, Veterans Admin. Medical Center, East Orange, NJ, 07019
Blood; 58(5):1053-1055 1981

Peripheral blood lymphocytes from normal donors and patients with chronic lymphocytic leukemia, B-cell type, were purified into T, helper T, and suppressor T lymphocytes by fluorescence-activated cell sorting using OKT3, OKT4, and OKT8 monoclonal antibodies. The max response of the purified subpopulations to stimulation by phytohemagglutinin (PHA) was determined by measuring the production of colonies when the stimulated cells were grown on agar. The helper T cells in normal and CLL patients were the most responsive to PHA stimulation, although the responsiveness of helper T cells to PHA was decreased in CLL. Purified CLL B cells responded minimally to PHA stimulation, but normal B lymphocytes did not. The abnormal response of CLL lymphocytes to PHA appears to be due to abnormal helper T cells, and, to a smaller extent, to the ability of CLL B lymphocytes to respond. (Author abstract) (17 Refs)

49. DIFFERENT RESPONSIVENESS OF COLONY-FORMING CELLS FROM NORMAL SUBJECTS AND CHRONIC MYELOID LEUKEMIA PATIENTS TO 12-O-TETRADECANOYLPHORBOL-13-ACETATE.

Pegoraro L, Bagnara G, Bonsi L, Biagini G, Garbarino G, Pagliardi GL
Instituto di Medicina Interna, Universita di Torino, 10126 Torino, Italy
Cancer Res; 41(12):5049-5051 1981

The effects of 12-O-tetradecanoylphorbol-13-acetate (TPA), a potent inducer of differentiation, upon the in vitro proliferation of normal bone marrow and chronic myeloid leukemia granulocyte-macrophage progenitor cells were investigated in the presence or absence of exogenous colony-stimulating activity. In the absence of colony-stimulating activity, no colony formation occurred, whereas its presence was followed by the appearance of colonies almost exclusively composed of macrophagic cells. However, both normal and leukemic granulocyte-macrophage progenitor cells showed a different response to TPA in terms of colony number and size. The number of colonies obtained from normal bone marrow was reduced to about 45% at three TPA concentrations (1 x 10(-8), 1 x 10(-10), and 1 x 10(-12) M). By contrast, the plating efficiency of leukemic granulocyte-macrophage progenitor cells was not significantly affected by 1 x 10(-10) and 1 x 10(-12) M TPA, but was strongly reduced by 1 x 10(-8) M. Moreover, TPA treatment did not significantly influence the size of colonies from normal bone marrow, while at 1 x 10(-10) and 1 x 10(-12) M, it remarkably increased that of the leukemic colonies. These different responses to TPA can be related to the abnormal sensitivity of chronic myeloid leukemia stem cells to factors regulating proliferation. (Author abstract) (24 Refs)

50. TETRAPEPTIDE H-TYR-HIS-LYS-LYS-OH INHIBITS PROLIFERATION OF NORMAL AND LEUKEMIC LEUKOCYTES IN VITRO (MEETING ABSTRACT).

Boranic M, Gabrilovac J, Pavelic J, Ferle-Vidovic A, Pavelic K, Skaric D, Skaric V
Rudjer Boskovic Institute, POB 1016, 41001 Zagreb, Yugoslavia
Exp Hematol; 9(Suppl9):27 1981

Many polypeptides isolated from various organs, tissues and cell cultures stimulate or inhibit cell growth and proliferation. Lysine seems to be an important component of these growth factors. In an attempt to define the active peptide sequences responsible for the effects on cell growth, we decided to study the effects of synthetic oligopeptides containing lysine on the proliferation of normal and leukemic WBC. A tetrapeptide containing tyrosine, histidine and 2 lysines was particularly active in our hands. Its mol wt is 574.67. Its effect was tested over a wide range of doses (from 0.1 to 1368 ng/ml) in short-term cultures of normal and phytohemagglutinin (PHA)-stimulated mouse spleen cells and human peripheral blood WBC, in the short-term cultures of mouse myeloid leukemia and human leukemic blasts, and in continuous cultures of mouse fibroblasts and HeLa cells. The substance diminished 3H-thymidine incorporation in PHA-stimulated cells, leukemic cells, and fibroblasts, but did not perceptibly affect non-stimulated cells and HeLa cells. The effect was dose-dependent. With leukemic

cells and fibroblasts, a paradoxical stimulatory effect was sometimes noted. (3 Refs)

51. ALKYL LYSOPHOSPHOLIPID INCUBATED HUMAN MACROPHAGES INHIBIT THE GROWTH OF AUTOLOGOUS SOLID TUMORS IN VITRO (MEETING ABSTRACT).

Berdel WE, Greiner E, Fink U, Stunkel K, Thiel E, Schwarzkopf G, Rastetter J
Department of Hematology Oncology, Tech University, Ismaningerst 22, 8000 Munich 80, W. Germany
Exp Hematol; 9(Suppl9):29 1981

Synthetic alkyl lysophospholipids (ALP) kill cells of human solid tumors selecti vely in vitro and inhibit the growth and metastasis of different syngeneic tumors in vivo. Furthermore, ALP render mouse macrophages cytotoxic in vitro and in vivo. As neoplastic cells lack alkyl cleavage enzymes these anti tumor actions might be based on a disturbance of phospholipid metabolism in tumor cell membranes. To establish the cytostatic activity of ALP incubated macrophages, mononuclear cells, have been obtained from peripheral blood via density gradients. Monocytes can be separated by a modified adherence procedure on membranes of polymeric fluorocarbon. By morphological, cytochemical staining and immunological characterization by monoclonal antibodies more than 92% of the remaining cells are monocytes. During cultivation for 10 days cells differentiate to adult macrophages. After 24-48 hr monocytes can be detached from the surface without loss in number or viability. Further cultivation on hydrophobic polymeric fluorocarbon prevents adherence for several days. Adult macrophages which were preincubated with different ALP inhibited the proliferation of autologous cells from different solid tumors. Cells of lung carcinomas and hypernephromas showed growth rates that were 30% and 10% of those of the controls. Inhibition correlates to macrophage:tumor cell ratio. Macrophages which have not been preincubated with ALP showed considerably lower effects on the tumor growth. (2 Refs)

52. DIFFERENCES IN THE REGULATION OF MURINE GRANULOCYTE AND MACROPHAGE GROWTH IN DIFFUSION CHAMBER CULTURES (MEETING ABSTRACT).

Page PL, Robinson SH, Charles A
Department of Medicine, Beth Israel Hospital, Boston, MA, 02215
Exp Hematol; 9(Suppl9):159 1981

As demonstrated under four sets of experimental conditions, murine macrophages do not respond to the same factors which influence granulocyte proliferation in diffusion chamber (DC) cultures: (1) When normal mouse bone marrow cells are cultured in DCs, a plateau in cell growth is observed after 7-10 days. During this plateau phase the same number of macrophages is observed regardless of the number of marrow cells placed in culture, whereas the plateau number of granulocytes varies in relationship to the number of marrow cells in the culture inoculum. (2) When DC cultures are established with marrow cells from donor mice which have been pretreated with 3 doses of cytosine arabinoside during the 14 hr before culture, macrophage growth is similar to that observed with cultures of normal marrow cells. In contrast, granulocyte growth in cultures derived from the marrows of drug-treated mice is enhanced by a factor of two. (3) When DC cultures of normal mouse marrow are implanted in host mice which have received 500 R whole body irradiation 24 hr earlier, growth of macrophages is unchanged whereas granulocyte growth is augmented, as compared to the findings with control cultures grown in unirradiated host mice. (4) When normal mouse bone marrow cells and murine acute myelogenous leukemic cells are cocultured in DCs, macrophage growth is unaffected whereas normal granulocyte growth is entirely abolished within a few days. In all four of these experimental situations granulocyte proliferation, but not macrophage growth, correlated well with the generation of colony forming units (CFU-C), as examined by serially harvesting DC cultures and assaying the cell contents in soft agar with L-cell conditioned medium as a source of colony stimulating activity. (no Refs)

53. CELL GROWTH FACTOR ACTIVITY: NEW QUANTITATIVE METHOD IN CELL CULTURE ASSAY.

Chen CH, Chen SC
Biochemistry Lab., Wilmer Ophthalmological Inst., Johns Hopkins Univ. Sch. Medicine, Baltimore, MD, 21205
Exp Cell Res; 136(1):43-51 1981

This quantitative method for the assay of growth factor activity in vitro was developed empirically. Based on the empirical equations, it seems logical to express the cell growth rate by cell duplication frequency, f, and the growth factor activity by the normalized cell growth rate: 1/(T-Tmin). The latter is recommended as the basis of defining an arbitrary unit of growth factor activity. For the in vitro growth factor activity assay, the quantitative method based on the empirical equations has two important features: (1) it appears to express linearly the growth factor concentration in assay, and (2) it is not affected by the initial cell counts and the length of cultivation. The view that these two features overcome the apparent defects associated with the expression systems that use the cell count ratio and net cell count is discussed. The quantitative method based on the empirical equations has been verified with experiments and compared with expression systems using the cell count ratio and net cell count. The experiments, which verified the quantitative method based on the empirical equations, were carried out with four selected cell lines: aortic endothelial cells, mouse 3T3 fibroblasts, Walker 256 carcinoma, and Chang's liver cells, using serum as the source of growth factor(s) for all cell lines. Vitreous, retinal extract, and Walker 256 carcinoma extract were also used for aortic endothelial cells. (Author abstract) (24 Refs)

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CELL AND SERUM DERIVED GROWTH EFFECTORS

1. EARLY CHANGES IN PHOSPHATIDYLINOSITOL AND ARACHIDONIC ACID METABOLISM IN QUIESCENT SWISS 3T3 CELLS STIMULATED TO DIVIDE BY PLATELET-DERIVED GROWTH FACTOR.

Habenicht AJ, Glomset JA, King WC, Nist C, Mitchell CD, Ross R (c/o Glomset) Regional Primate Res. Center SJ-50, Univ. Washington, Seattle, WA, 98195

J Biol Chem; 256(23):12329-12335 1981

We added platelet-derived growth factor to cultures of quiescent Swiss 3T3 cells to investigate early changes in lipid metabolism related to initiation of cell cycle traverse. In a series of experiments that focused on lipid degradation we added the growth factor to cells that had been prelabeled with myoinositol, glycerol, or arachidonic acid. We observed the following

HE 20.3173/2:

mitogen-dependent effects: a decline of radioactivity in cell phosphatidylinositol within 2 to 5 min that progressed to 25 to 50% during the 1st hr, a transient rise of radioactivity in cell diacylglycerol that peaked at 10 min, a gradual increase of radioactivity in monoacylglycerol in the medium, and a concomitant increase of radioactivity in medium-free fatty acid. In experiments that focused on lipid biosynthesis, we added the growth factor to cells and pulse-labeled them with radioactive precursors. We observed increased incorporation within 60 min of myoinositol into phosphatidylinositol, arachidonic acid into phosphatidylinositol, diacylglycerol, and phosphatidylethanolamine, and choline into phosphatidylcholine. These results support the possibility that action of platelet-derived growth factor on Swiss 3T3 cells leads to release of diacylglycerol from phosphatidylinositol, that some of the released diacylglycerol is hydrolyzed to monoacylglycerol and arachidonic acid, and that these lipid products are in part reconverted to phosphatidylinositol and other lipids. (Author abstract) (37 Refs)

2. DEMONSTRATION OF AN ANTIBODY AGAINST PLATELET-DERIVED GROWTH FACTOR.

Heldin CH, Westermark B, Wasteson A
Inst. Medical and Physiological Chemistry, Uppsala Univ., S-751 23 Uppsala, Sweden
Exp Cell Res; 136(2):255-261 1981

An antibody was raised in a rabbit against platelet-derived growth factor (PDGF), purified from human platelets. It recognized specifically 125I-labeled PDGF, as demonstrated by gel electrophoretic analysis of precipitated radioactivity. Addition of immunoglobulins from the immune serum to cell cultures together with PDGF inhibited the multiplication-stimulating activity of the growth factor. Further, multiplication-stimulating activity in crude platelet lysate was retained upon passage over a column of Sepharose-bound anti-PDGF; it was recovered after elution of the column with acid. A radioimmunoassay for PDGF was developed, permitting the determination of 5 ng PDGF/ml. Platelet factor 4 and beta-thromboglobulin, both being proteins of the platelet alpha-granule, did not cross-react in the radioimmunoassay for PDGF. The radioimmunoassay was utilized to examine the immunological relationship between PDGF and some other growth factors. The anionic growth factors previously demonstrated in platelets were found to be antigenically distinct from cationic PDGF. Similar results were obtained with epidermal growth factor (EGF) or fibroblast growth factor (FGF). In contrast, osteosarcoma-derived growth factor, a PDGF-like growth factor previously known as a product of cultured human osteosarcoma cells, showed some cross-reactivity in the radioimmunoassay for PDGF. It was concluded that osteosarcoma-derived growth factor (ODGF) has both physical-chemical and immunological properties in common with PDGF. (Author abstract) (20 Refs)

3. SIMILARITIES AND DIFFERENCES BETWEEN THE EFFECTS OF EPIDERMAL GROWTH FACTOR AND ROUS SARCOMA VIRUS.

Cooper JA, Hunter T
Tumor Virology Lab., Salk Inst., San Diego, CA, 92138
J Cell Biol; 91(3):878-883 1981

We have derived a line of A431 human tumor cells infected with Rous sarcoma virus (RSV). The infected cells contain the RSV-transforming protein, pp60src, which has characteristic tyrosine-specific protein kinase activity. As in other RSV-transformed cells, a 36,000-dalton protein is phosphorylated in RSV-infected A431 cells. Addition of epidermal growth factor (EGF) to the cells induces further phosphorylation of this protein. In contrast, this phosphoprotein is not detected in uninfected A431 cells, except when treated with EGF. Increased phosphorylation of the EGF receptor protein and of an 81,000-dalton cellular protein is dependent upon addition of EGF to the culture fluids, in both control and RSV-infected A431 cells. The results are discussed with reference to the similarities and differences between the tyrosine-specific protein kinases induced by RSV and activated by EGF. (Author abstract) (42 Refs)

4. THE ROLE OF CYTOPLASMIC MICROTUBULES IN THE REGULATION OF THE ACTIVITY OF PEPTIDE GROWTH FACTORS.

Friedkin M, Rozengurt E
Dept. Biology, Univ. California, San Diego, La Jolla, CA, 92093
Adv Enzyme Regul; 19:39-59 1981

The enhancement of DNA stimulation by peptide growth factors in colchicine-treated cultures of Swiss 3T3 mouse fibroblasts appears to be associated with disassembly of cytoplasmic microtubules. Other antitubulin agents (colcemid, vinblastine, podophyllotoxin, nocodazole, and maytansine) also potentiate the effects of growth promoting factors such as insulin and EGF. The cytoplasmic microtubules are disrupted by antitubulin agents at concentrations very similar to those required for depolymerization of mitotic spindle microtubules. In the presence of highly radioactive [3H]thymidine, DNA synthesis can be stimulated in quiescent mouse fibroblasts without completion of the cell cycle. This makes possible studies of the potentiating effects of colchicine during the prereplicative period of the cycle uncomplicated by effects of the antitubulin agent on the mitotic spindle. The enhancing effects of colchicine can be demonstrated with sparse cultures of 3T3 fibroblasts as well as with 3T6 cells. Maytansine and colchicine need not be present during the initial period of interaction of peptides with the cell to produce enhancement of DNA synthesis. The enhancement can be obtained after transient exposure of cells to fetal bovine serum. The potentiating effects of antitubulin agents such as nocodazole, podophyllotoxin, and maytansine can be easily reversed whereas the enhancement with colchicine persists despite washing of cells. The stimulatory effects of in sulin and EGF persist in cultures treated with antitubulin agents. The findings support the premise that after internalization the receptor-mitogen complex may remain active within endocytic vesicles when cells are exposed to colchicine. (Author abstract) (71 Refs)

5. TUMOR ANGIOGENESIS FACTOR (TAF) IN CULTURED CELLS DERIVED FROM CENTRAL NERVOUS SYSTEM TUMORS IN HUMANS.

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Dept. Neurosurgery, Neurological Inst. Faculty Medicine, Kyushu Univ., Fukuoka 812, Japan
Neurol Med Chir (Tokyo); 21(7):765-773 1981

Quantitative and qualitative assays were made of tumor angiogenesis factor (TAF) produced by cell lines derived from human CNS tumors. Supernatants were obtained from confluent cultures of five glioma cell lines (GCL), four primary cultures of meningiomas (PCM), one metastatic tumor cell line (MT), and one skin fibroblast cell line (SFC). TAF activities of supernatants corresponding to cell counts of 1.9-2.5 x 10(6) cells were assayed on chick chorioallantoic membrane. TAF activity was found in supernatants from 5/5 GCL, 3/4 PCM,

and the MT cultures. SFC produced no TAF activity. Analysis of TAF from one GCL showed that it was destroyed by ribonuclease or heating at 80 C for 30 min, but not by exposure to trypsin, deoxyribonuclease, or heating at 56 C for 30 min. TAF may therefore be a macromolecule related to tumor RNA. Exposure of GCL to 2,500 rads did not destroy TAF activity. TAF activity varied from one cell line to another, with primary or early passaged cultures producing greater activity than long-term cultures. (20 Refs)

6. A NOVEL GROWTH FACTOR FOR MOUSE MYELOID LEUKEMIC CELLS: PARTIAL PURIFICATION AND CHARACTERIZATION.

Komatsu K, Nakamura H, Akedo H
Dept. Biochemistry, Res. Inst., Center for Adult Diseases, Osaka, Japan
Nippon Ketsueki Gakkai Zasshi; 44(6):1124-1130 1981

A growth factor which promotes the multiplication of mouse myeloid leukemic cells (M1) was partially purified from mouse serum. The purification procedure included affinity chromatography using Blue Sepharose CL-6B and gel filtration of Bio-Gel P-150. Although the factor was co-fractionated with albumin upon the gel filtration, 71% of its activity was separated from albumin by the affinity column. The purified factor had a mol wt of about 65,000 daltons as determined by gel filtration at pH 7.4. Its isoelectric point was approximately 5.5. It was stable to heat (70 C for 30 min) and acid (pH 2.3) treatment, but was degraded by pronase. The dissociation at an acidic pH of the factor into a low mol wt form was not observed. Comparison of several properties of the factor with other established growth promoting serum factors suggests that it is a novel growth factor. (Author abstract) (24 Refs) (In JPN)

7. DIFFERENCES IN CYTOSOL FACTORS IN LIVER AND HEPATOMAS REVEALED BY REAL OR APPARE NT EFFECTS ON THE INCORPORATION OF [3H]THYMIDINE.

Barra R, Collin GR, Parsons J, Lea MA
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Int J Biochem; 13(12):1233-1240 1981

Effects of ethanol-extracted cytosol factors from rat liver and from Morris hepatomas on precursor incorporation was studied in hepatocytes and hepatoma cells. While precursor incorporation occurred, a portion of the effects may have been due to artifacts in the extraction procedure. Influence of cytosol fractions on incorporation of 3H-labeled thymidine and amino acids into nucleic acids and proteins was studied in hepatocytes from rats and HTC cells derived from the Morris hepatoma 7288C. Quantitative differences between tissues were seen most readily after precipitation of cytosol fractions with 45-70% ethanol. Effects were greater when cytosol fractions from normal or regenerating liver were used and incubations were made with hepatoma cells. An inhibitory action on the incorporation of (3H)thymidine into DNA, accompanied by increased incorporation into a residual protein fraction, was partially attributable to a requirement for more drastic conditions for DNA extraction in the presence of the cytosol fraction. Studies with (3H)lysine and (3H)leucine also resulted in increased incorporation into protein while long-term studies resulted in inhibition of cell proliferation. Results indicate a type of artifact not generally considered in the study of factors affecting (3H)thymidine incorporation. The data indicate changes in the cytosol which are a feature of neoplastic but not normal liver growth. (13 Refs)

8. LONG TERM CULTURE OF HUMAN T-CELLS IN THE PRESENCE OF T CELL GROWTH FACTOR (TCGF) FROM HUMAN TONSILS (MEETING ABSTRACT).

Fopp M, Lauber Ch, Senn HJ
Department of Internal Medicine, Division of Hematology and Oncology, Kantonsspital, St. Gallen, Switzerland
UICC Conference on Clinical Oncology, October 28-31, 1981, Lausanne, Switzerland. International Union Against Cancer 131 pp., 1981.

In the presence of supernatants of human WBC cultures, mitogen activated T-cells are growing in continued culture. The proliferative response of the cultured T-cells (CTC) is dependent on the source of the TCGF used. In contrast to human peripheral blood WBC (PBL) human spleen cells represent an excellent source of TCGF. Due to the more convenient availability, we were interested to look at the proliferative activity of supernatants from human tonsils. We sensitized mononuclears from human tonsils (5 x 10(6)/ml) with phytohemagglutinin (PHA). Supernatants were assayed for their proliferative activity of CTC by stimulation for 3 days during the 4th passage of a 12 day old T cell culture, followed by a 12 hr 3H deoxythymidine (TdR) pulse. Human tonsil cells produced high levels of TCGF with dose response curves similar to those observed with spleen cells. Some donors were low producers. Growth kinetics showed a five-fold increase of seeded cells every 72 hr. We conclude that mononuclears from human tonsils are able to produce TCGF upon stimulation with PHA. In contrast to spleen cells, there was a considerable difference in TCGF production between individual tonsil donors. Supernatants from human tonsils induced selective continuous proliferative growth of T-lymphocytes from mononuclears of healthy individuals as well as from leukemic patients during remission. (no Refs)

9. CHANGES OF CELLULAR MARKERS DURING DIFFERENTIATION OF HL-60 PROMYELOCYTES TO MACROPHAGES AS INDUCED BY T LYMPHOCYTE CONDITIONED MEDIUM.

Chiao JW, Freitag WF, Steinmetz JC, Andreeff M
Sloan-Kettering Inst. Cancer Res., 410 East 68th St., New York, NY, 10021
Leuk Res; 5(6):477-489 1981

Conditioned medium (CM) derived from allogeneic or phytohemagglutinin-stimulated lymphocytes was studied for its effect on cultures of the human promyelocytic cell line HL-60 from a patient with acute promyelocytic leukemia. Adherent cells in the proportion of 3-8% were noted beginning at 10-18 hr of incubation of the cultures with the CM; the proportion increased to 5-15% by 60 hr but decreased after 3 days. The CM-treated HL-60 cells differed from untreated cells in that they gradually underwent morphological changes indicative of differentiation. The earliest significant morphologic changes were seen after 3 days in culture; approx 25% of the cells were macrophage-like and 3% were monocyte-like. For culture days 1-3, the viability of the CM-treated cells (89%) was essentially the same as that of the cells (91%) before culturing with the CM. Continued culture with CM resulted in a further proportional decrease in promyelocytes, paralleled by a concomitant increase in monocyte- and macrophage-like cells. The findings suggested that CM-induced morphological maturation is caused by activated lymphocyte product. A decrease in HL-60 RNA content following CM culture seemed to be an early event in differentiation preceding morphological maturation. The proportion of proliferating cells decreased from 46% of the untreated cells to 13% after 7 days of CM incubation. During the

culture period the CM induced the development of membrane markers and functions that were absent in the untreated population. T lymphocytes were those most responsible for production of the maturation inducer in the CM; the inducer most likely stemmed from activated T lymphocytes. (37 Refs)

10. CHARACTERIZATION OF MONONUCLEAR BLOOD CELL-DERIVED DIFFERENTIATION INDUCING FACTORS FOR THE HUMAN PROMYELOCYTIC LEUKEMIA CELL LINE HL-60.

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JNCI; 67(6):1225-1230 1981

A search was made for physiologically produced differentiation inducing factor(s) (DIF) for the human promyelocytic leukemia cell line HL-60. Mononuclear blood cells, which stimulated with various mitogens, produced DIF, which induced differentiation of HL-60 into phagocytizing nitro blue tetrazolium reducing cells with the morphologic characteristics of granulopoietic or myelomonocytic cells. Induction of differentiation occurred equally well in serum-containing and serum-free media. Protein synthesis was necessary for elaboration of DIF, which seems to be of a protein nature inasmuch as it is destroyed by proteases. Gel chromatography showed that one or two species of DIF with apparent mol wt of 40,000 and 25,000 were produced, depending on the type of mitogen used. At least the 40,000-mol wt DIF was distinct from the colony stimulating activity (CSA), which was produced simultaneously. Our results suggested a role of lymphocytes and/or monocytes for modulation of myelomonocytic hematopoiesis not mediated by CSA. The physiologic importance remains, however, to be demonstrated. (Author abstract) (14 Refs)

HORMONES/ STEROIDS/ PROSTAGLANDINS AS GROWTH EFFECTORS

11. ESTROGEN INDUCED GROWTH OF HUMAN BREAST CANCER CELLS (MCF-7) IN ATHYMIC NUDE MICE IS ENHANCED BY SECRETIONS FROM A TRANSPLANTABLE PITUITARY TUMOR.

Welsch CW, Swim EL, McManus MJ, White AC, McGrath CM
Dept. Anatomy, Michigan State Univ., East Lansing, MI, 48824
Cancer Lett; 14(3):309-316 1981

The influence of pituitary hormones on growth of MCF-7 human breast tumor cells maintained in athymic nude mice was investigated. Sc injection of a pellet containing 17B-estradiol, and estrone in drinking water (0.5 mg/liter), resulted in sustained growth of the cells. Injection of a prolactin/growth hormone secreting rat pituitary tumor into the estrogen treated mice also caused growth of the tumor cells. No growth of tumor cells was observed in animals injected with pituitary tumor alone or in animals receiving neither pituitary tumor nor estrogen. Thus, rat pituitary tumor secretions enhanced the rate of in vivo MCF-7 tumor growth in estrogen treated animals, suggesting a synergism in their respective actions. (16 Refs)

12. ENDOCRINOLOGICAL ASPECTS OF BREAST PHYSIOLOGY (MEETING ABSTRACT).

Robyn C
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International Symposium on Endocrinology of Cystic Breast Disease, November 5-6, 1981, Turin, Italy Italian Society of Endocrinology; :A2 1981

Growth of the mammary gland is discontinuous; a first wave occurs at puberty under the influence of sex steroids, and the parenchymal breast tissue differentiates into a ductular-lobular-alveolar system. Growth slowly continues with each menstrual cycle up to the age of 30-35. A second major wave of growth takes place during pregnancy. Estrogens, progesterone and prolactin with variable relative potencies induce proliferation and differentiation of alveolar epithelial stem cells and control arborization of the ductular-lobular system. The interplay of the three hormones seems to be essential for optimal and balanced growth of the mammary tissue. From mid-pregnancy, the secretory differentiation of the alveolar stem cells is induced, predominantly under the influence of prolactin in synergism with metabolic hormones: this involves Messenger (m)-RNA synthesis for the production of milk proteins and enzymes. However, during pregnancy most of the secretory effects of prolactin are suppressed and lactation does not occur: in vitro, progesterone suppresses the prolactin effects of casein m-RNA synthesis. After delivery, progesterone, estrogens and placental lactogen abruptly drop and prolactin induces full secretory activity and milk release. Oxytocin is required for milk ejection. Prolactin and oxytocin are released by neuroendocrine reflexes initiated by suckling. Prolactin does not appear to be essential for maintaining lactation. In mothers nursing for 2 yr or more, hyperprolactinemia persists for 15-18 mo; it is associated with infertility due to anovulation or corpus luteum deficiency. The decline in serum prolactin levels seen in the mothers after 18 mo of lactation is not associated with a decline in milk yield, at least when considering the amount of milk per feeding. Finally, the sequence of the endocrine events controlling full differentiation of the breast tissue and the timing of their occurrence seem to be of decisive importance in the resistance of the mammary epithelium to carcinogenic agents. (no Refs)

13. NEUROHORMONAL AND CHEMOSENSITIVITY OF HUMAN MELANOMA COLONY FORMING UNITS IN SOFT AGAR (MEETING ABSTRACT).

Meyskens FL
University of Arizona, Tucson, AZ
Third NCI-EORTC Symposium on New Drugs in Cancer Therapy, October 15-17, 1981, Brussels, Belgium European Organization for Research on Treatment of Cancer; :A20 1981

We measured the effect of a series of non-steroidal hormones (follicle stimulating hormone (FSH), luteinizing hormone (LH), melanocyte stimulating hormone (MSH), human chorionic gonadotropin (HCG), melatonin, nerve growth factor (NGF)) on human melanoma colony forming cells in soft agar from biopsies of 20 patients with metastatic melanoma. A complex array of responses were demonstrated, suggesting that a diversity of hormonal phenotypes are present. Melanoma colony forming units in soft agar from biopsies of 50 patients with metastatic melanoma demonstrated marked heterogeneity in chemosensitivity to nine chemotherapeutic drugs. Reduction in survival of colony forming units below 38% at one-tenth the pharmacologically achievable one hr concentration x time (our operational definition of chemosensitivity) was obtain ed in only 19% of 200 in vitro trials and was generally identical whether

patients had been exposed to prior chemotherapy or not, suggesting that melanoma colony forming units are inherently resistant to presently available chemotherapeutic drugs. The soft agar assay was 86% accurate (25 of 29 cases) for identifying drugs to which the tumor was resistant in vivo and 63% accurate (12 or 19 trials) for identifying drugs to which the tumor was clinically sensitive when mixed responses were included as responses. In contrast, if mixed responses were classified as progressive disease, then the accuracy for identifying sensitivity fell to 42% (8 of 19 trials). These investigations furnish a quantitative description of the chemosensitivity of human metastatic melanoma colony forming units and demonstrate that neurohormones can modulate melanoma colony forming units. Additionally, these studies can be regarded as a useful step towards the development of an in vitro chemosensitivity test for human melanoma and provide an operational quantitative basis for further exploration of in vitro directed therapy in metastatic neoplasms and classification of melanoma by neurohormonal responses. (no Refs)

14. LONG-TERM CULTURES IN POLYCYTHEMIA VERA AS PROOF OF DEPENDENCE ON ERYTHROPOIETIN.

Heilmann E, Holzknecht A, Fahrenkrug H, Essers U
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Blut; 43(1):51-56 1981

Erythropoietin (EP)-dependence of erythroid colony proliferation was studied in eight patients with polycythemia vera (PV). Growth of colonies on methylcellulose ended after 3 wk in the absence of EP. With the addition of EP culture growth rose sharply in the first 2 wk, dropped in the 3rd wk, and then maintained a constant rate up to 9 wk. Apparently erythropoiesis in PV is EP-dependent. (18 Refs) (In GER)

CYCLIC NUCLEOTIDE/ POLYAMINES/ NUCLEOTIDES AS GROWTH EFFECTORS

15. PLASMA MEMBRANE-MEDIATED EFFECTS OF EXTRACELLULAR PH ON THE GROWTH OF NEUROBLASTOMA CELLS.

van der Saag PT, Feyen A. Miltenburg-Vonk W, De Laat SW
Hubrecht Lab., International Embryological Inst., 3584 CT Utrecht, The Netherlands
Exp Cell Res; 136(2):351-358 1981

Mouse neuroblastoma cells (Neuro-2A) were cultured at pH values between 6.5 and 8.0. Acidification of the culture medium resulted in a reversible inhibition of growth. To explore the possibility that the effect of extracellular pH on growth is mediated by the plasma membrane, plasma membrane microviscosity, cellular cAMP levels and adenylate cyclase activities were measured after exposing the cells to various pH levels. Upon lowering the extracellular pH from 7.6 to 6.5, plasma membrane microviscosity decreases within a few hr to 50%. Concomitantly cellular cAMP levels increase more than three-fold, while ade nylate cyclase activity is enhanced three-fold as well, accompanied by a small increase in Km of the enzyme. All these effects are reversible. These results are compatible with the idea that the growth-regulatory role of the extracellular pH is mediated at the level of the plasma membrane, probably by altered membrane-adenylate cyclase interactions. (Author abstract) (65 Refs)

16. THE ACTION OF ADENOSINE ANALOGS ON PC12 CELLS.

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NIH, NICHD, LDN, Building 6, Room 1A08, Bethesda, MD, 20205
J Neurochem; 37(6):1431-1439 1981

PC12 cells, a nerve growth factor-responsive clone of rat pheochromocytoma, contain a membrane-bound adenylate cyclase, which can be activated by adenosine analogs. The characteristics of the cyclase response indicate the presence of stimulatory adenosine receptors. Adenosine analogs also produce a marked increase in the ornithine decarboxylase levels of the cells, and the characteristics of this response suggest that it is linked to the adenylate cyclase-stimulatory adenosine receptors. The ornithine decarboxylase response elicited by 5'-N-ethylcarboxamideadenosine (NECA), a potent stimulatory adenosine analog, is synergistic with that produced by nerve growth factor. Differentiation of the cells with nerve growth factor, however, does not substantially alter either the response of cyclase to the adenosine analog or the magnitude of the adenosine-evoked ornithine decarboxylase response. Treatment of the cells with NECA produces an increase in the phosphorylation of a specific non-histone nuclear protein. While causing little or no morphological alteration by itself, NECA is synergistic with nerve growth factor in producing neurite outgrowth in PC12 cells. NECA does not cause an induction of acetylcholinesterase in the cells, nor does it appear to affect the induction of this enzyme by nerve growth factor. (Author abstract) (34 Refs)

EXOGENOUS AGENTS AFFECTING DIFFERENTIATION

17. INHIBITORS OF CELL DIVISION REVERSIBLY MODIFY HEMOGLOBIN CONCENTRATION IN HUMAN ERYTHROLEUKEMIA K562 CELLS.

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NIH, Building 10, Room 9N-307, Bethesda, MD, 20205
Blood; 58(6):1236-1239 1981

The human leukemia K562 cell line can be induced by 20 uM hemin to reversibly accumulate embryonic and fetal hemoglobins without any change in the rate of cell division. When we reduced the rate of cell division by glutamine starvation or addition of hydroxyurea, the cells increased by 10-fold the basal hemoglobin level of 0.3-0.5 pg Hb/cell. The combined effects of hemin and inhibitors of cell division permitted K562 cells to attain levels of hemoglobin (26-34 pg Hb/cell) close to that found in normal red cells. This superinduction was reversible and cells could be recycled indefinitely. Furthermore, electrofoc using experiments show that the three primary hemoglobin species produced by these cells (Hb Gower 1, Hb Portland, and fetal Hb), were induced, or reinduced, synchronously by inhibitors of cell division but asynchronously by hemin. Differing effects of hemin and inhibitors of cell division were observed in the absence of irreversible differentiation and suggest different molecular mechanisms controlling globin gene expression. (Author abstract) (15 Refs)

18. REGULATION OF ERYTHROID CELL DIFFERENTIATION BY HAEMIN.

Bonanou-Tzedaki SA, Sohi M, Arnstein HR
Dept. Biochemistry, King's Coll. London, Strand, London WC2R 2LS, England
Cell Differ; 10(5):267-279 1981

The effects of up to 100 uM exogenous hemin on the growth and differentiation of fractionated immature erythroid cells derived from the bone marrow of anemic rabbits are described. New Zealand white male rabbits (1.5-2.5 kg body wt) were made anemic by sc injections of 2.5% neutralized phenylhydrazine hydrochloride and 1 mM mercaptoethanol. Different erythroid cell fractions showed considerable variation in extent and rate of growth and in the response to erythropoietin or hemin. Cultures containing 50 uM hemin showed changes in cell distribution representative of a net increase in the number of orthochromatic cells. In contrast to erythropoietin-treated cultures, the increase was not paralleled by an increase in number of early erythroid cells. After 17 hr of culture in the presence of 20, 50, or 100 uM hemin, cells exhibited DNA synthesis at 74%, 55%, and 22% of control culture levels, respectively. Addition of hemin to late erythroid cells (polychromatic and orthochromatic cells) had little effect on their already reduced DNA synthetic activity. Comparison of the effects of hemin and inhibitors of nucleic acid synthesis on cell survival and differentiation, and the specificity of the hemin effect, are discussed. (33 Refs)

19. MITOGENIC AND CO-MITOGENIC PROPERTIES OF HEMIN.

Stenzel KH, Rubin AL, Novogrodsky A
Dept. Biochemistry, Rogosin Kidney Center, Cornell Univ. Medical Coll., New York, NY, 10021
J Immunol; 127(6):2469-2473 1981

We report here that hemin, an agent known to induce cellular differentiation in Friend erythroleukemia cells, induces mitogenesis in human T cells and enhances mitogenic responses to supraoptimal concentrations of Con A. Mitogenesis induced by suboptimal or optimal concentrations of Con A is not affected by concentrations of hemin that markedly potentiate responses to supraoptimal amounts of Con A. Maximum mitogenic responses are reached 4 to 5 days after initiation of the cultures. The mitogenic response is macrophage dependent, whereas enhancement of responses to supraoptimal concentrations of Con A is not. Indeed, a greater degree of enhancement is observed after removal of adherent cells. Responses to other mito gens (phytohemagglutinin, wheat germ agglutinin, and sodium periodate) are also enhanced by hemin. Compounds that are metabolically or structurally related to hemin are neither mitogenic nor co-mitogenic. Protoporphyrin IX, which is nonmitogenic, is rendered mitogenic by the chemical insertion of iron to form hemin. Hemin does not affect Con A binding to lymphocytes. These findings indicate that hemin is a macrophage-dependent T cell mitogen with the unusual and remarkable property of overcoming the inhibition of mitogenesis induced by high concentrations of Con A. Since hemin has a limited number of metabolic effects, it provides a useful probe for determination of molecular events associated with lymphocyte activation. (Author abstract) (28 Refs)

20. USE OF FLOW CYTOMETRY AND COLONY FORMING ASSAYS IN MONITORING DIFFERENTIATION OF HUMAN PROMYELOCYTIC LEUKEMIA CELLS (HL-60) (MEETING ABSTRACT).

Andreeff M, Lu L, Swartwout S, Broxmeyer HE
Memorial Sloan-Kettering Cancer Center, New York, NY
VIII Conference on Analytical Cytology and Cytometry, May 19-25, 1981, Wentworth-by-the-Sea, New Hampshire Memorial Sloan-Kettering Cancer Center; 1981

In this study, the effect of a variety of agents on two different clones of HL-60 cells (diploid(2.0C) and aneuploid (3.7C) DNA stemlines) was investigated. Morphology, acridine orange flow-cytometry (FC) for cellular DNA and RNA content, and the capacity to form colonies in semi-solid medium were employed as markers of differentiation. Agents used included dimethyl sulfoxide (DMSO 1.3%), 12-O-Tetradecanoylphorbol-13-acetate (TPA 10-8M), Cis-Retinoid Acid (RA 10-6M), colony-stimulatory factor containing conditioned medium from placental cells (PL-CSA 10% v/v) and the human monocyte- like cell line GCT (GCT-CM 10% v/v), E-type prostaglandin (PGE1 10-6M), and acidic isoferritin (10-8M). Differentiation as determined by morphology was induced by DMSO, TPA and RA. This was substantiated by flow cytometric DNA/RNA measurements on day 6 of the experiment showing decrease of proliferation and RNA content: the diploid cells had a decrease in RNA-Index of 50.9, 25.6 and 38.9%, respectively, the aneuploid cells of 48.9, 41.9 and 25.2%, respectively. TPA induced death on day 6 in approx 80% of both cell lines, DMSO in 79.9% of diploid and 39.2% of aneuploid cells, RA in 61.6% of diploid, and 67.4% of aneuploid cells. The number of cells in S-phase decreased from 29.0% in the diploid line to 5% (DMSO) and 14% (RA), and from 31.0% in the aneuploid line to 10.0% (DMSO) and 25.0% (RA), respectively. FC demonstrated no change in RNA and proliferation for GCT-CM in diploid and aneuploid cells, but a slight loss of RNA in PL-CSA (6.6%), PGE (13.8%), and isoferritin (6.8%) not seen by morphology, with unchanged proliferation and viability. Changes induced by DMSO, TPA and RA were coincident with a decrease in colony and cluster formation, but no significant changes were seen for the other agents. The three independant assays used corroborate the finding that DMSO, TPA and RA are potent inducers of differentiation. Determination of cellular DNA and RNA allows are to quantitate early molecular events related to differentiation on a single cell basis. (no Refs)

21. EXPRESSION OF NORMAL MONOCYTE-MACROPHAGE DIFFERENTIATION ANTIGENS ON HL60 PROMYELOCYTES UNDERGOING DIFFERENTIATION INDUCED BY LEUKOCYTE-CONDITIONED MEDIUM OR PHORBOL DIESTER.

Todd RF, Griffin JD, Ritz J, Nadler LM, Abrams T, Schlossman SF
Div. Tumor Immunology, Sidney Farber Cancer Inst., Harvard Medical Sch., Boston, MA, 02115
Leuk Res; 5(6):491-495 1981

The acquisition of normal, nonpolymorphic surface membrane antigens Mo1 and Mo2 was demonstrated in a significant proportion of HL60 human promyelocytic cells that were induced to differentiate by WBC-conditioned medium or by the phorbol ester 12-O-tetradecanoylphorbol-13-acetate. Mo2 antigen was specific for late monocyte-macrophage differentiation and was expressed by peripheral blood monocytes and cultured macrophages but not by earlier myeloid forms including promyelocytes. The Mo1 antigen was found on mature granulo-

cytes and null cells as well as on monocytes and macrophages. (8 Refs)

OTHER RELATED STUDIES

22. MECHANISM OF GROWTH STIMULATION OF L1210 CELLS BY 2-MERCAPTOETHANOL IN VITRO. ROLE OF THE MIXED DISULFIDE OF 2-MERCAPTOETHANOL AND CYSTEINE.

Ishii T, Bannai S, Sugita Y
Div. Biochemistry, Tsukuba Univ. Sch. Medicine, Sakura, Niihari, Ibaraki, 305, Japan
J Biol Chem; 256(23):12387-12392 1981

The mechanism of the growth-promoting action of 2-mercaptoethanol on mouse lymphoma L1210 cells in vitro has been studied. The normal line of L1210 is deficient in its capacity to take up cystine, and this limits the growth of the cells in normal culture media containing cystine. In the presence of 2-mercaptoethanol, the cells obtain a high capacity to utilize cystine in the medium. The reaction of 2-mercaptoethanol with cystine produces a mixed disulfide of 2-mercaptoethanol and cysteine. The mixed disulfide is taken up by the cells mainly via the L system, a transport system for neutral alpha-amino acids such as leucine. The mixed disulfide within the cells is rapidly reduced to produce cysteine and 2-mercaptoethanol which escapes rapidly into the medium and reacts with cystine again. With the aid of this cyclic action of 2-mercaptoethanol, the cells are able to utilize cystine constantly. (Author abstract) (29 Refs)

23. CHARACTERISTICS OF TEMPERATURE SENSITIVITY OF VARIOUS GYNAECOLOGICAL MALIGNANT TUMOR CELLS UNDER HYPERTHERMIC CONDITIONS (39-42C) IN VITRO (MEETING ABSTRACT).

Udagawa Y, Nozawa S, Tsukazaki K, Ohta H, Tsutsui F, Kurihara S
Department of Obstetrics and Gynaecology, School of Medicine, University of Keio, Tokyo, Japan
Obstetrics and Gynaecology: 8th Asian Congress, October 25-31, 1981, Melbourne, Australia. Asia and Oceania Federation of Obstetricians and Gynaecologists 352 pp., 1981.

The difference in temperature sensitivity between normal human diploid and various gynecological malignant tumor cells in vitro was studied. These malignant cells were human endometrial adenocarcinoma (SNG-M), cervical epidermoid carcinoma (SKG-1), uterine leiomyosarcoma (SKN) and malignant melanoma cells (CAK). In this study, the inhibitory effect of cell proliferation and the degenerative changes on cell morphology of these cells for about four wk at high temperatures (30-42C) were investigated. Results were: (1) The malignant cells were more heat sensitive at and over 40C than normal cells, which continued to grow until 42C. In other words, permissive temperatures to cell growth of these cells were approx 2-3C lower than those of normal cells. Especially at 42C and/or higher, they failed to grow further and degenerated with karyorrhexis within a few days. (2) There were some differences in heat sensitivity between these cells of different histological origin. (3) No specific distribution pattern of chromosomal number was found at any temperature in all cases. It was clarified that there was a significant difference in heat sensitivity between normal and various gynecological malignant tumor cells of different histological origin. Our results further support that hyperthermia might be useful for treatment of gynecological malignancy. (no Refs)

24. STIMULATION OF NA INFLUX, NA-K PUMP ACTIVITY AND DNA SYNTHESIS IN QUIESCENT CULTURED CELLS.

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Adv Enzyme Regul; 19:61-85 1981

A central problem in cell regulation is to elucidate the biochemical basis of the metabolic activation seen in quiescent cells stimulated to proliferate. Recent evidence has shown that an increase in the rate of Na influx is one of the earliest events in the stimulation of quiescent cells into DNA synthesis and cell division. This phenomenon has been demonstrated in a variety of fibroblastic cells and it is elicited by serum, as well as by purified growth factors. The stimulation of Na influx is apparently sufficient to increase the activity of the Na-K pump of the plasma membrane. The activity of this enzyme is sensitive to small fluctuations of cellular Na, as shown by using the selective Na ionophore monensin, which increases cellular Na and Na-K pump activity. A number of recent findings suggest that the influx of Na ions into the cell can signal later events of cell proliferation, specifically the initiation of DNA synthesis. The evidence supporting this notion is as follows: (a) the rate of Na entry rapidly increases in a variety of quiescent fibroblastic cells stimulated to proliferate by serum or purified growth factors; (b) a decrease in Na entry by lowering the Na concentration in the extracellular medium or by inhibiting the uptake of Na by amiloride markedly blocks the initiation of DNA synthesis promoted by serum or growth factors; (c) the neurohypophyseal nonpeptide vasopressin, the potent tumor promoter TPA, the amphipathic polypeptide melitin and the channel-forming antibiotic amphotericin B stimulate Na ion influx and trigger the initiation of DNA synthesis, acting synergistically with other growth factors; (d) certain cells transformed by oncogenic viruses show an increased Na flux as compared with their normal counterparts. These findings suggest that Na influx may signal the initiation of mitogenesis in quiescent cells. (Author abstract) (84 Refs)

25. RECIPROCAL INDUCTION OF DIFFERENTIATION IN MIXED CULTURE OF NEUROBLASTOMA AND GLIOMA CELLS.

Allin EP
Institut de Physiologie et de Biochimie, Universite Louis Pasteur, Strasbourg, France
Cell Mol Biol; 27(2/3):291-293 1981

Co-culture of rat glioma and mouse neuroblastoma cells results in a mutual differentiation of both neurons and glia regardless of the serum concentration and the different species involved. This differentiation is stable and cultures can be maintained for several months. (Author abstract) (10 Refs)

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CELL AND SERUM DERIVED GROWTH EFFECTORS

1. ISOLATION OF A PLURIPOTENT CELL LINE FROM EARLY MOUSE EMBRYOS CULTURED IN MEDIUM CONDITIONED BY TERATOCARCINOMA STEM CELLS.

Martin GR
Dept. Anatomy, Univ. California, San Francisco, CA, 94143
Proc Natl Acad Sci USA; 78(12):7634-7638 1981

This report describes the establishment directly from normal preimplantation mouse embryos of a cell line that forms teratocarcinomas when injected into mice. The pluripotency of these embryonic stem cells was demonstrated conclusively by the observation that subclonal cultures, derived from isolated single cells, can differentiate into a wide variety of cell types. Such embryonic stem cells were isolated from inner cell masses of late blastocytes cultured in medium conditioned by an established teratocarcinoma stem cell line. This suggests that such conditioned medium might contain a *growth factor* that stimulates the proliferation or inhibits the differentiation of normal pluripotent embryonic cells, or both. This method of obtaining embryonic stem cells makes feasible the isolation of pluripotent

HE 20.3172/2

cell lines from various types of noninbred embryos, including those carrying mutant genes. The availability of such cell lines should make possible new approaches to the study of early mammalian development. (Author abstract) (22 Refs)

2. MOUSE EMBRYONIC TRANSFORMING GROWTH FACTORS RELATED TO THOSE ISOLATED FROM TUMOR CELLS.

Twardzik DR, Ranchalis JE, Todaro GJ
Lab. Viral Carcinogenesis, NCI, Frederick, MD, 21701
Cancer Res; 42(2):590-593 1982

Growth factors with the two characteristic properties of sarcoma growth factor, the ability to stimulate anchorage-independent growth of normal mouse or rat fibroblasts and the ability to compete with 125I-labeled epidermal growth factor for receptor binding, can be isolated from 12-to 13-day-old normal mouse embryos of various strains. Two size classes of the transforming factor from embryo cells can be isolated with apparent mol wt of 20,000 and 10,000. The lower-mol-wt factor has been purified several hundred-fold and has the same properties as the peptide with a mol wt of 10,000 produced by mouse sarcoma virus-transformed 3T3 cells. Whole-mouse embryos contain approx 10% as much sarcoma growth factor per g of tissue as do the murine sarcoma virus-transformed cells; how it is distributed among the embryonic tissues remains to be determined. (Author abstract) (14 Refs)

3. EFFECT OF TUMOR-RELATED FACTORS ON THE IN VIVO AND IN VITRO COLONY-FORMING ABILITY OF NORMAL MOUSE MARROW CELLS.

Pessina A, Eridani S, Brambilla P, Cattoretti G, Marocchi A, Mocarelli P
Dept. Pathology, Hosp. Desio, Desio (Milan), Italy
Neoplasma; 28(5):541-548 1981

The effect of normal mouse marrow preincubation with Ehrlich tumor fluid (EAF) and Ehrlich tumor cell-conditioned medium (ET-CM) was investigated both on the in vivo assay for pluripotential stem cells (CFU-S) and on the in vitro assay for granulocytic-macrophagic progenitors (CFU-C). A significant reduction of the number of CFU-S was found when bone marrow cells were incubated with EAF. The morphological analysis showed a relative increase in the granulocytic type colonies besides an absolute increase in undifferentiated ones and an absolute decrease in erythroid colonies. The in vitro data show a decrease in CFU-C production, by stimulation with standard Colony Stimulating Factor (CSF), when the marrow cells are preincubated with EAF and ET-CM. When standard CSF (obtained from L-cells) is used at the higher concentrations, the CFU-C in the ET-CM treated marrow increases to the control values. EAF and ET-CM incubation also affects the CFU-C differentiation by increasing the number of pure granulocytic clones. The experi mental data were discussed in order to explain a hypothesis about the activity of tumor-related factors. (Author abstract) (16 Refs)

4. GROWTH FACTORS PRODUCED BY MALIGNANT CELLS.

Keski-Oja J, Vaheri A
Suomen Akatemia, Helsingin yliopiston virusopin laitos, 00290 Helsinki 29, Finland
Duodecim; 97(17):1383-1393 1981

The growth of normal cells is carefully controlled mostly by hormones, by hormone-like growth factors (GFs) and by extracellular substances, while malignant cells are in certain respects autonomous, less influenced than normal cells by body fluids and GFs from in vitro cultures. They do not react typically with other cells and are less inhibited in growth density. The malignant cell has the capacity to secrete its own GFs, which can even affect the secreting cell itself. It is not known what other target cells this 'autocrinic' secretion may have, but normal cells in the immediate vicinity tend to be involved with them. Some tumor-derived phenotypes and growth patterns are augmented when added to normal cell cultures. Tumor cells also secrete proteolytic enzymes and vascular change accelerating factors, contributing in turn to invasive, expansive growth in vivo. A study of these 'metastasizing' factors is important both for understanding the mechanism of malignant growth and for clarifying related possibilities for diagnosis and treatment. Subjects discussed are the histological characteristics of malignancy, polypeptide GFs secreted by normal tissues, sarcoma GFs, the transforming GF of human tumors, malignant-growth accelerating factors derived from cancer cells, MSA-like GFs, NGF-like GFs of melanoma cells, and the GFs of hematopoietic cells. (50 Refs) (In FIN)

5. STIMULATION OF GROWTH OF TUMOR CELLS IN CULTURE BY THEIR OWN GROWTH FACTOR(S) (MEETING ABSTRACT).

Nagao Y, Nishikawa K
Department of Biochem, Kanazawa Medical University, Ishikawa, Japan
Proceedings of the Japanese Cancer Association 40th Annual Meeting, October 1981, Sapporo, Japan. The Association 415 pp., 1981.

We have already found growth factors, which stimulate the growth of BALB/3T3 cells, in the extract of rat Rhodamine fibrosarcoma (RdFs). In this report, we will present the evidence that the extract from RdFs contains the factor(s) which stimulate the growth of cultured primary RdFs cells. The cells were cultured in the medium comprising a mixture of F12 and L15 (1:1)supplemented with 0.25% control serum (CS). The cell number was counted on day 4. When RdFs extract was added at protein concentration of 100 ug/ml, the cell number increased to 4 x above the control. Maximal growth in the presence of the tumor extract and 0.25% CS was higher than that achieved with optimal concentration of CS. This factor(s) could not be substituted by other growth factors, such as epidermal growth factor, fibroblast growth factor and hydrocortisone. (no Refs)

6. EFFECT OF THE SUSPECTED TUMOR PROMOTERS SACCHARIN, CYCLAMATE, AND PHENOL ON NERVE GROWTH FACTOR BINDING AND RESPONSE IN CULTURED EMBRYONIC CHICK GANGLIA.

Ishii DN
Dept. Pharmacology, Cancer Res. Center, Coll. Physicians and Surgeons of Columbia Univ., New York, NY, 10032
Cancer Res; 42(2):429-432 1982

The suspected bladder tumor promoters saccharin and cyclamate reversibly inhibited nerve growth factor-induced neurite outgrowth in embryonic chick sensory ganglia, and active concentrations of these artificial sweeteners inhibited binding of 125I-labeled mouse submaxillary gland nerve growth factor as well. The skin tumor promoter phenol also reversibly inhibited neurite outgrowth, while comparable concentrations of the nonpromoting but structurally related compounds benzene and fluorobenzene did not. However, in contrast to findings with saccharin and cyclamate, phenol had little, it any, effect on

binding of radioactive nerve growth factors. (Author abstract) (58 Refs)

7. MONOCLONAL ANTIBODIES AGAINST RECEPTOR FOR EPIDERMAL GROWTH FACTOR INDUCE EARLY AND DELAYED EFFECTS OF EPIDERMAL GROWTH FACTOR.

Schreiber AB, Lax I, Yarden Y, Eshhar Z, Schlessinger J
Dept. Chemical Immunology, Weizmann Inst. Science, 76100 Rehovot, Israel
Proc Natl Acad Sci USA; 78(12):7535-7539 1981

Mice were immunized with human epidermoid carcinoma cells (A-431 cell line) that possess an unusually high number of membrane receptors for epidermal growth factor (EGF). Spleen cells from these mice were fused with NSI cells, a non-secreting murine myeloma. The immunoglobulins secreted by the obtained hybridomas were screened for specific binding to A-431 cells and selected according to their ability to inhibit the binding of radiolabeled EGF to the membrane of A-431 cells. Several antibodies secreted by cloned hybrid lines were found to inhibit the binding of radiolabeled EGF to membrane receptors of living A-431 cells, human foreskin fibroblasts, and mouse 3T3 fibroblasts and also to membrane preparations from A-431 cells. These monoclonal antibodies induced the early and delayed biological effects mediated by EGF. Like EGF, the antibodies induced morphological changes in A-431 cells and enhanced the phosphorylation of endogenous membrane proteins in membranes from these cells. They also stimulated DNA synthesis in human foreskin fibroblasts. These observations support the notion that the biological information of the EGF-receptor complex resides in the membrane receptor. Furthermore, the antibodies offer a powerful tool to study the structure, processing, and mode of action of EGF receptors. (Author abstract) (34 Refs)

8. MICROTUBULE STABILIZATION BY TAXOL INHIBITS INITIATION OF DNA SYNTHESIS BY THROMBIN AND BY EPIDERMAL GROWTH FACTOR.

Crossin KL, Carney DH
Dept. Human Biological Chemistry and Genetics, Div. Biochemistry, Univ. Texas Medical Branch, Galveston, TX, 77550
Cell; 27(2):341-350 1981

Several observations have suggested that microtubule depolymerization might act as a regulator of cell proliferation. To determine whether microtubule depolymerization is required for growth-factor-induced initiation of DNA synthesis, we treated serum-free cultures of mouse embryo cells with taxol to stabilize their microtubules and measured the initiation of DNA synthesis by thrombin and epidermal growth factor (EGF). Pretreatment of quiescent cultures of mouse embryo cells with 10 ug/ml taxol inhibited up to 60% of the thrombin-stimulated and 47% of the EGF-stimulated DNA synthesis. This inhibition was dose-dependent for taxol concentrations from 0.3 to 20 ug/ml. Control experiments showed that taxol did not simply affect uptake of nucleotides, glucose or amino acids, nor did it nonspecifically affect protein synthesis, cell morphology or cell viability. Taxol did not affect binding and internalization of 125I-thrombin or 125I-EGF indicating that the drug does not alter receptor number, affinity or distribution after growth factor binding. Taxol also did not affect the proteolytic activity of thrombin. Thus it appears that the inhibitory effects of toxol are mediated by a direct effect of taxol on microtubules. To determine at which point microtubule stabilization was interrupting the initiation signal, we added taxol at various times after addition of thrombin or EGF. Taxol addition during the first 8 hr after growth factor addition inhibited initiation, but after 8 hr had little if any effect. These results confirm that taxol was not nonspecifically affecting transport or metabolism required for DNA synthesis and indicate that thrombin and EGF may initiate cell proliferation through a gradual microtubule depolymerization or rearrangement that is necessary to commit cells to a replicative cycle. (Author abstract) (48 Refs)

9. FUNCTIONAL RECEPTORS FOR EPIDERMAL GROWTH FACTOR IN HUMAN OSTEOSARCOMA CELLS.

Shupnik MA, Tashjian AH
Lab. Toxicology, Harvard Sch. Public Health, Boston, MA, 02115
J Cell Physiol; 109(3):403-410 1981

Epidermal growth factor (EGF) is a 53-amino acid polypeptide isolated from mouse submaxillary glands. The clonal line A151B1 of human mouse osteosarcoma cells G-292 responded to treatment with EGF by increased prostaglandin E2 (PGE2) production. Binding of 125I-EGF to cells had a 6 hr plateau phase; leupeptin (50 ug/ml) prevented decreases in bound EGF, which typically followed the plateau phase in untreated cells. EGF binding was of high affinity (Kd = 1.9 x 10(-9) M) and was to a single class of non-interacting binding sites. PGE2 production was increased three-fold by 48 hr of EGF treatment, but c onstant EGF was required to maintain the response. DNA synthesis did not increase in EGF-treated cells, nor did medium or intracellular cyclic AMP increase. Binding and biological actions of EGF on G-292 cells were the same as those in whole mouse bone for the tested responses, suggesting that G-292 cells could serve as a model system for studies of EGF action on osseous tissue. (22 Refs)

10. PLATELET-DERIVED GROWTH FACTOR INCREASES PROSTAGLANDIN PRODUCTION AND DECREASES EPIDERMAL GROWTH FACTOR RECEPTORS IN HUMAN OSTEOSARCOMA CELLS.

Shupnik MA, Antoniades HN, Tashjian AH
Lab. Toxicology, Harvard Sch. Public Health, Boston, MA, 02115
Life Sci; 30(4):347-353 1982

Human platelet-derived growth factor (PDGF) stimulated the production of prostaglandin E2 (PGE2) by G-292 cells, a clonal line of human osteosarcoma cells. Half-maximal stimulation occurred with 9 ng/ml PDGF and maximal stimulation, three-fold above control values, occurred with 40 ng/ml of the protein. Treatment of G-292 cells with 40 ng/ml PDGF also reduced the binding of iodinated epidermal growth factor (EGF) to EGF receptor on G-292 cells. The effect was time-dependent, and EGF binding was reduced to 60% of control by 24-48 hr. PDGF did not, however, compete directly for binding to the EGF receptor. The effects of PDGF and EGF on increased PGE2 production appeared to be additive at all concentrations tested, indicating that they may act through a common pathway, but not via the same membrane receptors. (Author abstract) (27 Refs)

11. PLATELET-DERIVED GROWTH FACTOR (PDGF) ENHANCES THE GROWTH OF MOLONEY SARCOMA VIRUS-INDUCED TUMORS IN MICE.

Inglot AD, Inglot O
Lab. Tumor Virology, Dept. Tumor Immunology, Inst. Immunology and Experimental Therapy, Polish Acad. Sciences, Czerska 12, 53-114 Wroclaw, Poland
Arch Immunol Ther Exp (Warsz); 29(4):431-437 1981

The intramuscular (im) administration of bovine or human platelet derived growth factor (PDGF) had a marked potentiating effect on the growth of Moloney sarcoma virus (MSV)-induced tumors in BALB/c mice. PDGF had the tumor-promoting-activity when administered im on the side of inoculation of MSV 2 days after the virus, and subsequently every 2nd day for 2-3 wk. The extent of MSV-induced tumor promoting activity of PDGF was age and sex-related. In view of the recently described antagonism in action between growth factors and interferons it is suggested that both families of hormones play an important role in controlling the neoplastic proliferation. (Author abstract) (16 Refs)

12. HUMAN ADHERENT CELL CONTACT-MEDIATED MODULATION OF NORMAL MYELOID COLONY FORMATION.

Spitzer G, Verma DS, Beran M, Zander AR, McCredie KB, Dicke KA
Dept, Developmental Therapeutics, Univ. Texas System Cancer Center, M. D. Anderson Hosp. and Tumor Inst., 6723 Bertner Ave, Houston, TX, 77030
JNCI; 68(1):35-42 1982

The effects of coincubation of normal nonadherent bone marrow cells on adherent monolayers created from human peripheral blood mononuclear cells or marrow cells were investigated. Nonadherent marrow cells were coincubated for 4 hours with peripheral blood adherent cells at ratios of adherent cells to marrow cells of 2:1 to 5:1. This coincubation suppressed subsequent neutrophilic agar colony growth but not eosinophilic growth. Further studies suggested that this suppression was a cell-cell-mediated process and not secondary to soluble factors. However, coincubation on marrow adherent cells caused increased neutrophilic colony recovery. The possible in vivo relevance is discussed. (Author abstract) (26 Refs)

13. CLONE SIZE POTENTIAL AND SENSITIVITY TO COLONY-STIMULATING ACTIVITY: DIFFERENTIATION-LINKED PROPERTIES OF GRANULOCYTE-MACROPHAGE PROGENITOR CELLS.

Francis GE, Berney JJ, Bodger MP, Bol SJ, Wing MA, Hoffbrand AV
Dept. Haematology, The Royal Free Hosp., Pond St., Hampstead, London NW3 2QG, England
Stem Cells; 1(2):124-139 1981

Clone size potential and sensitivity to the specific granulocyte-macrophage regulator colony-stimulating activity (CSA) were studied. Properties of normal granulocyte-monocyte progenitor cells and the difference in linked properties were investigated. The results show that clone size potential and sensitivity to CSA are properties of granulocyte-macrophage clone-forming cells linked in an organized fashion. Four different techniques were used to minimize the possibility of obtaining apparent linking of the two properties merely due to production of artefacts during the separation procedure or to co-purification of CSA-resistant cells with a different population which have large clone size potential. Bone marrow cells from normal donors were used. The techniques enabled the use of clonogenic cells with different equilibrium density centrifugation, adherence to microcarrier beads, osmotic lysis, and fluorescence-activated cell sorting. Mean clone size is directly related to the amount of CSA required to stimulate 50% of the cells to clone formation. Results showed that the linking of clone size potential and sensitivity to CSA is not confined to acute myeloid leukemia clonogenic cells since it occurred within normal granulocyte-monocyte progenitor populations. The differences in the two properties reflect the position of the normal progenitors in the granulocyte-monocyte maturation hierarchy. (21 Refs)

14. IN VITRO INDUCTION OF BONE MARROW CELL DIFFERENTIATION BY PARTIALLY PURIFIED COLONY STIMULATING FACTORS (CSF) FROM PSK-SERUM (MEETING ABSTRACT).

Sato M, Osawa T
Fac of Pharm Sci, University of Tokyo, Tokyo, Japan
Proceedings of the Japanese Cancer Association, 40th Annual Meeting, October 198 1, Sapporo, Japan. The Association 415 pp., 1981.

Our previous studies have shown that an ip injection of PSK (500 mg/kg) temporarily elevates serum CSF level either in normal or in tumor bearing mice. We obtained two active fractions eluting at different NaCl concentrations in ion-exchange chromatography which induced the proliferation and differentation of bone marrow cells in vitro. Although the colonies and colony forming cell in semisolid culture system induced by these factors were morphologically different, the cells in both cultures were tightly adhesive and non-specific esterase positive, and phagocytized latex beads showing monocyte-macrophage properties. Further investigation on biological functions of these cells: accessory role on immune system and cytotoxic effect on cancer immunology will be presented. (no Refs)

15. ISOLATION AND CHARACTERIZATION OF A COLONY-STIMULATING FACTOR (CSF) FROM THE URINE OF FIBROSARCOMA-BEARING MICE (MEETING ABSTRACT).

Shikita M, Tsuneoka K, Bessho M, Hirashima K
National Institute Radiology Science, Chiba-shi 260, Japan
Proceedings of the Japanese Cancer Association, 40th Annual Meeting, October 1981, Sapporo, Japan. The Association 415 pp., 1981.

It has been reported that transplantation of the fibrosarcoma (NFSA) to C3H mice resulted in marked increase of mature granulocytes in peripheral blood of the animals, and that granulocyte-macrophage colony-stimulating factor was secreted from the tumor cells cultured in vitro. In the present study, it was found that the tumor-bearing mice excreted a large amount of CSF in urine. The CSF was purified by diethylaminoethyl-cellulose column chromatography, isoelectrofocusing, gel-filtration on Ultrogel ACA 34, and finally by Concanavalin A (Con A) Sepharose affinity chromatography. It showed an isoelectric point at pH 3.0, and an apparent mol wt of 80,000. The results suggest that the CSF is a sialoglycoprotein resembling human urine CSF. In a preliminary experiment, the CSF stimulated the growth of human myeloid stem cells in semi-solid agar culture. This is inactive from human cells. (no Refs)

16. T-CELL GROWTH FACTOR-MEDIATED PROLIFERATION OF LYMPHOCYTES FROM A T-CHRONIC LYMPHOCYTIC LEUKAEMIA PATIENT LACKING MITOGEN AND ALLOANTIGEN RESPONSIVENESS.

De Vries JE, Vyth FA, Mendelsohn J
Div. Immunology, The Netherlands Cancer Inst., Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands
Clin Exp Immunol; 43(2):302-310 1981

Peripheral blood lymphocytes (PBL) of chronic lymphocytic leukemia of T cell origin (T-CLL), which were unresponsive to mitogens and had negligible stimulator and responder capacities in mixed lymphocyte cultures (MLC), were induced to proliferate by human T cell growth factors (TCGF). Long-term T-CLL PBL cultures were obtained in the presence of TCGF. T-CLL PBL obtained from a female patient (30 y r old) showed no 3H-thymidine (3HTdR) incorporation when stimulated with various concentrations of phytohemagglutinin (PHA), Concanavalin A, or pokeweed mitogen. The T-CLL PBL exhibited greatly reduced responder and inducer capacities (12% and 14% that of normal sheep RBC rosette-forming cells, respectively). TCGF-1 had the strongest growth-promoting activity and was used as a standard (1.0 growth-promoting unit). TCGF-1,-2, and-3 contained 0.52, 0.29, and 0.33 growth-promoting units, respectively. PHA stimulated proliferation of T-CLL cells in the presence of TCGF-1. Both TCGF-3 and-4 also induced and maintained T-CLL PBL proliferation in the presence of PHA, but at considerably lower rates than those produced in the presence of TCGF-1 and-2. In MLC, addition of TCGF-4 resulted in a four-fold increase in 3H-TdR incorporation in T-CLL PBL after stimulation with lymphocytes from a normal donor (C). Lymphocytes from donor C showed an 80-fold increase in 3H-TdR incorporation when cultured with T-CLL PBL in the presence of TCGF-4. The results indicated that the lack of response to mitogens and alloantigens of the T-CLL PBL can be attributed to the failure of these cells to produce TCGF upon activation. (36 Refs)

17. T-CELL GROWTH FACTOR (INTERLEUKIN 2) CONTROL OF T-LYMPHOCYTE PROLIFERATION: POSSIBLE INVOLVEMENT IN LEUKEMOGENESIS.

Gillis S, Mertelsmann R, Moore MA
Basic Immunology Program, Fred Hutchinson Cancer Res. Center, 1124 Columbia St., Seattle, WA, 98104
Transplant Proc; 13(4):1884-1890 1981

Isolation of murine and human leukemic T-cell lines that produce exceptionally high quantities of interleukin 2 (IL-2), and screening of freshly isolated patient-derived human leukemic blasts for IL-2 production and response capabilities, are described. An IL-2 microassay was used to screen fractions of biochemically manipulated, conditioned medium for the capacity to promote sustained in vitro proliferation of murine cytotoxic T-lymphocytes. Two leukemic cell lines, murine LBRM-33 and human Jurkat-FHCRC, produced large quantities of IL-2 upon stimulation with phytohemagglutinin (PHA). Both of the cell lines bear conventional T-cell markers and phenotypic markers indicative of immature cell type. Analysis of leukemic blast cell response of mononuclear blood cells from patients with various leukemias revealed consistent IL-2 production and response patterns. All patients who exhibited high levels of deoxynucleotidyl transferase (TdT) in leukemic blood cells showed a small proliferation increase in response to PHA stimulation but produced exceptionally high levels of IL-2. The response to added purified human IL-2 and PHA was atypical in most patients, in that incorporation of 3H-thymidine decreased in the majority of TdT+ leukemias when compared to PHA-stimulated proliferation. Such a finding was in marked contrast to results o btained with normal lymphocytes. IL-2 production and use responses and TdT expression in leukemic cells are discussed. (17 Refs)

18. MECHANISMS CONTROLLING TCGF PRODUCTION BY CLONED SUBLINES OF EL-4 AZGR IN RESPONSE TO STIMULATION BY ANTI-THY-1 ANTIBODY.

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J Immunol; 128(1):296-301 1982

The effect of xenogeneic anti-Thy-1 antibody on T cell growth factor (TCGF) production by T lymphoma cell lines has been examined as a model system for T cell activation. EL-4 G12 (a cloned subline of the producer EL-4 azgr cell line) produced TCGF when stimulated by a high concentration of anti-Thy-1, but none was induced by low concentrations of anti-Thy-1. Large amounts of TCGF were produced when these cells were cultured with Fc-receptor positive (FcR+) accessory cells. TCGF production by EL-4 G12 showed dose response kinetics similar to TCGF production by anti-Thy-1 stimulated purified normal spleen T cells. Goat anti-rabbit IgG (GaRIG) and Protein A substituted for this accessory helper effect, but neither FcR+ cells nor Protein A worked when (Fab')2 anti-Thy-1 was used instead of IgG anti-Thy-1. Anti-T-200 monoclonal antibody inhibited anti-Thy-1-induced TCGF production by EL-4 G12 and accessory cells. Phorbol myristic acetate and lymphocyte-activating factor also substituted in part for the accessory cell help. The data suggest there are at least two different accessory cell help mechanisms in anti-Thy-1-induced TCGF production, anti-Thy-1-bound membrane aggregation either by GaRIG, Protein A or FcR, and a LAF-dependent mechanism. (Author abstract) (29 Refs)

19. STIMULATORY ACTIVITY FOR HUMAN PLURIPOTENT HEMOPOIETIC PROGENITORS PRODUCED BY A HUMAN T-LYMPHOCYTE CELL LINE.

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Stem Cells; 1(2):73-80 1981

A human T-cell line (Mo) constitutively produces stimulatory activities for human pluripotent stem cells. Nine bone marrow samples from eight individuals were cultured with 5% phytohemagglutinin-stimulated leukocytes (PHA-LCM) or 5% Mo-conditioned medium (Mo CM) obtained from various passages. Three bone marrow specimens were cultured with partially purified erythroid-potentiating activity (EPA). The frequency of mixed colonies observed with various Mo CM preparations was almost always higher than the frequency observed from PHA-LCM. The cellular composition of mixed colonies grown with PHA-LCM varied from that seen with Mo CM. Mixed colonies grown in the presence of Mo CM developed a large eosinophilic component in addition to a small number of neutrophils and a larger number of erythroblasts. Megakaryocytes were not observed in colonies grown with Mo CM. The size of colonies grown in the presence of Mo CM was smaller than the size observed with PHA-LCM. Erythroid burst formation was at best increased to levels obtained with PHA-LCM, while the planting efficiency of target cells (CFU-C) was consistently higher with crude Mo CM preparation. No differences were observed comparing the stimulatory effect of the original material with the boiled preparations, indicating

that these stimulatory activities are quite heat stable. A similar requirement for erythropoietin was observed in cultures stimulated by crude or purified Mo CM or PHA-LCM. The most potent stimulator of mixed colonies tested was a preparation of EPA partially purified from Mo CM. (22 Refs)

20. INCREASE AND INHIBITION OF PRE-B-CELL PROLIFERATION IN CULTURE, BY T CELLS.

Paolucci P, Layward L, Hayward AR, Rapson NT, Soothill JF
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Clin Exp Immunol; 43(2):336-341 1981

The effect of T cells on in vitro proliferation of pre-B cells (PBC) from marrow of patients with acute lymphatic leukemia (ALL) was studied. ALL-PBC occur in greater numbers in the marrow and proliferate in vitro more rapidly than PBC from normal individuals. PBC and B cells were obtained from 23 children (4-14 yr old, av age 8.5 yr) 1-6 mo after the end of successful treatment for ALL. The number of PBC was abnormally low in ALL patients during treatment but rose to abnormally high levels within 2 mo after withdrawal of treatment. Proliferation of PBC in vitro was decreased after removal of T cells by E-rosette formation. Proliferation was restored after addition of normal heterologous T cells. T cells from ALL patients (either syngeneic or heterogeneic) had a greater stimulatory effect on PBC proliferation than did normal T cells. T cells that had been exposed to 10 ug concanavalin A/ml suppressed PBC proliferation. Results indicate that T cells can either increase or reduce proliferation of PBC from bone marrow of children with ALL. This suggests that nonspecific effects of T cells are required for proliferation of PBC in vitro. The in vivo significance of T cell effects on PBC proliferation is uncertain. (12 Refs)

HORMONES/ STEROIDS/ PROSTAGLANDINS AS GROWTH EFFECTORS

21. EFFECTS OF GLUCOCORTICOID HORMONES ON CELL PROLIFERATION IN DIMETHYLHYDRAZINE-INDUCED TUMOURS IN RAT COLON.

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Dept. Anatomy, Monash Univ., Clayton, Victoria, 3168, Australia
Virchows Arch [Cell Pathol]; 38(2):247-251 1981

Adrenocortical hormones have previously been shown to influence cell proliferation in many tissues. In this report, their influence on cell proliferation in the colonic crypt epithelium and in colonic adenocarcinomata is compared. Colonic tumor cell proliferation was found to be retarded following adrenalectomy and this retardation was reversible by administration of hydrocortisone, or by administration of synthetic steroids with predominantly glucocorticoid activity. Tumor cell proliferation in adrenalectomized rats was not promoted by the mineralocorticoid hormone aldosterone. Neither adrenalectomy, nor adrenocortical hormone treatment, significantly influenced colonic crypt cell proliferation. (Author abstract) (21 Refs)

22. CELL CYCLE-SPECIFIC GLUCOCORTICOID GROWTH REGULATION OF A HUMAN CELL LINE (NHIK 3025).

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Inst. Biophysics, Univ. Trondheim, 7034 Trondheim-NTH, Norway
J Cell Physiol; 109(3):489-496 1981

Cells, synchronized by mitotic selection, were exposed to dexamethasone (DEX) throughout the cell cycle. Cells exposed during the first half of G1 had lengthened cell cycle time, and most of the prolongation occurred in that phase. Exposure during other phases was ineffective. The growth inhibition was reversible, but did not extend to subsequent cell cycles where there was no exposure to DEX. (23 Refs)

23. LACK OF EFFECT OF DIETARY FAT ON THE GROWTH AND ESTROGEN SENSITIVITY OF THE MT-W9B TRANSPLANTABLE MAMMARY TUMOR.

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Nutr Cancer; 3(1):27-34 1981

Effects of diets containing low- (LF: 0.5%) or high (HF: 20%) fat on the growth and estrogen responsiveness of the MT-W9B mammary tumor in Wistar-Furth rats were studied. Tumor growth was not significantly affected by HF or LF diet. Tumor growth was also unaffected in either group by ovariectomy (OVx), sham OVx, or by OVx with estradiol supplementation. Dietary fat intake did not alter the sensitivity of the uterus to estrogen. Progesterone receptor (PR) levels were slightly elevated in tumors from sham-OVx rats fed a HF diet compared those fed a LF diet; this increase was not statistically significant. Responses of PR to OVx or to OVx with estrogen supplementation were not altered by dietary fat levels. PR levels were decreased in OVx rats to 16% and 14% of control levels in LF and HF groups, respectively, and increased 16-fold and 13-fold, respectively, in OVx rats given estradiol. In the same tumors, estrogen receptor levels were not significantly altered by either dietary or hormonal manipulation. PR levels were elevated in uteri from sham-OVx rats fed a HF diet compared to those fed a LF diet when expressed on a picomole/uterus basis, but this increase was not statistically significant when expressed on a per mg basis. Dietary fat did not appear to affect the response of uterine PR to estrogen manipulation. Dietary effects on estrogen receptors are described. The results suggested that dietary fat does not influence the sensitivity of normal or neoplastic tissue to estrogen action. (37 Refs)

24. RELATIONSHIP OF WEIGHT TO THE PROMOTION OF BREAST CANCER AFTER MENOPAUSE.

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Dept. Epidemiology, Inst. Social Medicine, Utrecht, The Netherlands
Nutr Cancer; 2(4):237-240 1981

Epidemiologic and experimental evidence for a relation between high body wt and breast cancer is reviewed. A survey of the female population including 23,500 women 50 yr or older gave evidence for the initiation-promotion concept of breast cancer. Body wt and height were measured at each screening examination. Estrogen receptors (ER) were measured in 180/258 patients with breast cancer that was diagnosed during the survey. ER were found in 117 patients. The determination of ER-status of cancers has provided evidence that promotion in ER-positive cases is favored by obesity and inhib-

ited by leanness. Estrogen-dependent breast cancer can be promoted by extra-ovarian estrogens from adipose tissue. The effect of wt and obesity is probably mediated through a menopausal estrogen mechanism. In lean postmenopausal women, clonal selection is thought to lead to a preponderance of ER-negative cells in cancer. Initiation of breast cancer is probably unrelated to obesity. Induction of breast cancer may be rare after menopause, but existing tumors grow if they receive stimuli to which they are sensitive. If wt reduction would prolong the free interval in breast cancer (until recurrence of secondaries), it would probably also be a means of controlling ER-positive breast cancer in its preclinical stages. (23 Refs)

25. CHARACTERIZATION OF UNIQUE NUCLEAR ESTROGEN BINDING COMPONENT IN NEOPLASTIC LEYDIG CELL OF MICE (MEETING ABSTRACT).

Sato B, Maead Y, Matsumoto K
The Third Department of Internal Medicine, Osaka University School of Medicine, Osaka, Japan
Proceedings of the Japanese Cancer Association, 40th Annual Meeting, October 1981, Sapporo, Japan. The Association 415 pp., 1981.

The unique nuclear estrogen binding component (EBC) in one of mouse Leydig cell tumors was examined. This EBC was found to be associated with nuclei even in this absence of estrogen. The steroid specificity of this EBC was quite similar to that of putative cytosolic estrogen receptor. The affinity for estrogens, however, seemed to be regulated by temperature and divalent ion; that is, raising temperature to 30C and/or introducing EDTA in buffer systems resulted in significant and reversible reduction of Kd from 10-8M to 10-9M. The possibility of the artificial redistribution of EBC during subcellular fractionation was eliminated by demonstrating a lack of estrogen receptor in the cytosol. This EBC was found to be relatively resistant to micrococcal nuclease, RNase or 0.4M KCl treatment, but quite sensitive to trypsin digestion. The stimulation of host animals with *estrogens accelerated tumor growth,* suggesting that this unique EBC is a biologically active mediator of estrogen action. (no Refs)

26. THE ROLE OF PROSTAGLANDINS IN THE DEVELOPMENT OF MALIGNANT MELANOMA IN HAMSTERS.

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Medical Clinic No 1, Central Military Hosp., Bucharest, Romania
Prostaglandins Med; 7(4):321-325 1981

Prostaglandin (PG) treatment was tested in animal malignant melanoma. A deficiency of PG (especially of group E) could be an important factor in the genesis and evolution of malignant melanoma. Two groups of hamsters bearing malignant melanomas were used; the test group (16 females, 2 males) received 1 ml PGE2 solution (in phosphate buffer at a concentration of 50 nanog/ml) daily for 12 days. The PG solution was injected directly into the tumor. Control animals (14 females) received daily injections of phosphate buffer under the same conditions. Tumor growth slowed and stopped in the PG-treated group but progressed in the control group. Ten days after stopping treatment,the tumors in the PG-treated group began to grow as before. Well-differentiated tumors with discrete infiltration of adjacent tissues but no macroscopic metastases were found. Microscopic examination showed malignant melanomas with a low density of atypical cells. Compensation for PG deficit would be a logical way of medically treating malignant melanoma. (26 Refs)

EXOGENOUS AGENTS AFFECTING DIFFERENTIATION

27. THE TUMOR PROMOTER, 12-O-TETRADECANOYLPHORBOL-13-ACETATE ACCELERATES KERATINOCYTE DIFFERENTIATION AND STIMULATES GROWTH OF AN UNIDENTIFIED CELL TYPE IN CULTURED HUMAN EPIDERMIS.

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Exp Cell Res; 137(1):155-167 1982

Studies were conducted to determine the effects of the mouse skin tumor promoter 12-O-tetradecanoylphorbol-13-acetate (TPA) on cultured human epidermal cells for comparison with known effects on mouse keratinocytes. In contrast to its effect on mouse cells, TPA did not stimulate human epidermal cell DNA synthesis. TPA stimulated differentiation in human keratinocytes resulting in sloughing of many cells by the 3rd day after exposure. Quantitative assays revealed that 50% of the TPA-exposed population was composed of cornified cells as opposed to 8% in untreated controls. A morphologically distinct cell type (TT cell) emerged after TPA treatment which was triangular in shape, did not stratify, appeared to proliferate rapidly and at most TPA concentrations became the predominant cell type within 1-2 wk. Cultures composed predominantly of TT cells formed few cornified envelopes, grew well in the absence of TPA and formed colonies at low cell input. In contrast to its effect on keratinocytes, TPA enhanced TT colony formation three-four fold and decreased the doubling time of TT cells. Studies were perfor med to determine the origin of TT cells. Immunofluorescent staining indicated that TT cells lacked the keratinocyte antigens keratin, pemphigus and pemphigoid. Tonofilaments and desmosomes were not seen by electron microscopy. The lack of both melanosomes and standard histochemical DOPA oxidase staining indicated that TT cells were probably not of melanocyte origin. Tests used to identify Langerhans cells were negative. Whereas TT cells, as well as dermal fibroblasts, yielded positive immunofluorescence with antibodies to vimentin, TT cells gave a weak histochemical leucine aminopeptidase reaction, while the reaction of fibroblasts exposed to TPA was strong. Treatment of human dermal fibroblasts with TPA did not yield TT cells. The endothelial cell antigen Factor VIII-associated protein was absent by immunofluorescence. These results suggest that the primary effect of TPA on cultured human epidermis is to accelerate terminal differentiation in the keratinocyte population and to stimulate growth of an as yet unidentified cell type. (Author abstract) (25 Refs)

28. DECREASED SYNTHESIS OF HIGH-MOLECULAR-WEIGHT GLYCOPEPTIDES IN HUMAN PROMYELOCYTIC LEUKEMIC CELLS (HL-60) DURING PHORBOL ESTER-INDUCED MACROPHAGE DIFFERENTIATION.

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Cancer Res; 42(2):484-489 1982

The human promyelocytic leukemia cell line, HL-60, synthesized a class of high-mol-wt (MW 5,000 to 7,000), N-linked glycopeptides as the major class of protein-bound carbohydrates. Small glycopeptides (MW 2,500 to 3,500), typical of

most mammalian cells except RBC, represented a minor component in these cells. The large glycopeptides were labeled efficiently with fucose, glucosamine, and galactose but only poorly with mannose. They were found not to be glycolipids, glycosaminoglycans, or mucin-type glycopeptides and were not susceptible to exoglycosidases, but they were partially degraded by endo-beta-galactosidase. These characteristics are similar to those of the large glycopeptides synthesized by RBC, by another human myeloid leukemia cell line (K562), and by human and murine teratocarcinoma cells. High-mol-wt glycopeptides predominated on another human myeloid leukemia cell line KG1, but they were expressed at low levels on both a human monocytic leukemia cell line (THP-1) and a human T-lymphoblastoid cell line (Jurkat). when HL-60 cells were induced to differentiate into macrophage-like cells with phorbol esters, the proportion of large glycopeptides decreased, and the production of small glycopeptides predominated. This shift was observed within the first several hr after exposure to phorbol esters and was temporally related to the acquisition of adherent properties by the induced cells. In contrast, when HL-60 cells were ind uced to differentiate into granulocytes by dimethyl sulfoxide, hypoxanthine, or retinoic acid, they continued to synthesize glycopeptides similar to uninduced cells. Human peripheral blood granulocytes synthesized primarily large glycopeptides, whereas monocytes and lymphocytes synthesized mostly small glycopeptides. These results indicate that the synthesis of high-mol-wt glycopeptides is a property of human myeloid leukemia cell lines and that it persists throughout myeloid differentiation. A proportionate decrease in the synthesis of these large glycopeptides is a part of the differentiation program for monocytes and macrophages. (Author abstract) (34 Refs)

29. INDUCTION OF DIFFERENTIATION OF HL-60 CELLS BY DIMETHYL SULFOXIDE: EVIDENCE FOR A STOCHASTIC MODEL NOT LINKED TO THE CELL DIVISION CYCLE.

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Cancer Res; 42(2):445-449 1982

Induction of differentiation of the human promyelocytic leukemia cell line HL-60 by dimethyl sulfoxide (Me2SO) was analyzed to determine the relationship between exposure time of the inducer and cell cycle. A minimum incubation time of 12 hr with Me2SO was required in order to induce differentiation in a small but significant proportion of cells. These expressed differentiation markers (morphology, phagocytosis, and nitroblue tetrazolium reduction) up to 12 hr after Me2SO was removed from the medium. For periods beyond 12 hr and as long as 120 hr of contact of HL-60 cells with the inducing agent, a linear rise in the percentage of differentiated cells was observed. The sensitivity to Me2SO of HL-60 cells synchronized by double thymidine block was examined and was found to be similar to that of nonsynchronized cells. The effect of Me2SO was not altered when incubated with cells at different phases of the cell cycle. Finally, even nonproliferating cells were sensitive to the inducing effect of Me2SO. The data are consistent with a stochastic model of induction to differentiation without having any linkage to the cell cycle. (Author abstract) (29 Refs)

30. CHANGES IN ACID PROTEOLYTIC ACTIVITY DURING DIFFERENTIATION OF MURINE ERYTHROLEUKEMIC CELLS.

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Cell Differ; 10(6):309-315 1981

Changes in proteolytic activity of the murine erythroleukemic cell line 745 (MEL cells) were followed during chemically induced differentiation. In uninduced cells, proteolytic activity peaked on day 2 of culture and was retained thereafter. Two days after addition of 2% (vol/vol) dimethylsulfoxide (DMSO), 4 milliM hexamethylene bisacetamide, or 1.5 milliM butyric acid, protease activity rose to an even higher level than that in undifferentiated cells. However, enzymic activity markedly declined after 3 days; the decline paralleled cellular differentiation and was accompanied by Hb accumulation. The direct connection between induction of differentiation and decreased protease activity was supported by the observation that clone R-1, which is resistant to DMSO-induced differentiation, showed no change in protease activity after exposure to DMSO. Proteolytic activities measured before and after differentiation were of similar pH optima and showed similar sensitivity to pentamidine isethionate, antipain, phenylmethyl sulfonyfluoride, and pepstatin; however, tosylamide-phenylmethyl-chloromethyl ketone inhibited 60% of activity in uninduced cells vs 93% of activity in differentiated cells. Migration of proteolytic activity together with the lysosomal marker alpha-mannosidase on colloidal silica gradients indicated that the activity is located in the lysosomes. (23 Refs)

31. MODIFICATION OF ALPHA-FETOPROTEIN AND ALBUMIN SYNTHESIS BY DIMETHYLSULFOXIDE IN AN ESTABLISHED LINE OF MOUSE HEPATOMA CELLS.

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Cell Struct Funct; 6(3):295-300 1981

The effect of dimethylsulfoxide (DMSO) on synthesis of albumin (ALB) and alpha-fetoprotein (AFP) was studied in mouse epithelioid tumor cells of line BW1, derived from transplantable BW7756 mouse hepatoma. BW1 cultures were exposed to DMSO (final concentration 0, 0.5, 1.0, or 3.0%) for 4 days. ALB and AFP in cell lysates and culture supernatants were quantified using specific rabbit antisera. AFP and ALB accounted for 0.51 and 0.53%, respectively, of total cell-associated protein (TCAP) in BW1 cultures not treated with DMSO. Intracellular (IC) and extracellular (EC) ALB and AFP were increased by exposure of cells to DMSO. The ALB percentage of TCAP increased with increasing concentrations of DMSO. Max AFP percentage of TCAP was produced by 0.5% DMSO; above this level the AFP fraction declined slightly, although in all DMSO treated cultures the AFP fraction was approx 60% greater than in control cultures. Treatment with 1% DMSO increased EC AFP by 156% and EC ALB by 214%. Increases in IC AFP and ALB evidently were not due to inhibition of transport mechanisms. Apparently ALB synthesis is dependent on DMSO concentration, while AFP synthesis, once initiated, is independent of DMSO concentration. DMSO treatment reduced the final number of cells in culture by 57% with 1% soln and by 84% with 3% soln. Results indicate that in vitro expression of growth properties correlates

with an enhanced capacity for production of differentiated gene products. (15 Refs)

32. MEMBRANE PROTEIN REDISTRIBUTION DURING DIFFERENTIATION OF CULTURED HUMAN ERYTHRO LEUKEMIC CELLS.

Hunt RC, Marshall LM
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Mol Cell Biol; 1(12):1150-1162 1981

Human erythroleukemic (K562) cells incubated with 0.05 milliM hemin undergo differentiation along the erythroid pathway. RBC proteins synthesized before and after such induction, morphology of the cells and localization of membrane proteins at the cell surface were examined. In the presence of hemin, cells continued to proliferate; after a 24-48 hr delay they started to synthesize Hb. During differentiation, no changes in major cell surface proteins were detected by lactoperoxidase-catalyzed iodination. Specific immune precipitation showed no change in synthesis of spectrin, the major cytoskeletal protein of the mature RBC. While no major changes in cell surface proteins were detected, scanning and transmission electron microscopy revealed marked changes in the morphologic organization of the cell membrane. A process similar but not identical to the enucleation observed in erythroid differentiation in vivo occurred: a smooth-surfaced cell approx 10 micron in diameter divided from the nucleus-containing part of the cell. Transmission electron microscopy showed that with the exception of ribosomes, these reticulocyte-like cells contained no organelles, but they contained much of the parent cell's Hb, spectrin, and glycophorin. (32 Refs)

33. NK CELL SENSITIVITY OF THE LEUKEMIC K 562 CELLS; EFFECT OF SODIUM BUTYRATE AND HEMIN INDUCTION.

Dokhelar MC, Testa U, Vainchenker W, Finale Y, Tetaud C, Salem P, Tursz T
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J Immunol; 128(1):211-216 1982

Two differentiation inducers, hemin and sodium butyrate, have been studied for their effect on the NK sensitivity of human leukemic K 562 cells. When cultivated in the presence of 0.1 mM hemin, K 562 cells increased their hemoglobin synthesis. However, their susceptibility to NK lysis remained unaffected. On the other hand, sodium butyrate, which is known as a more potent differentiating agent, consistently reduced the NK sensitivity of K 562 cells and simultaneously induced the appearance of several differentiation markers. The effect of sodium butyrate on NK sensitivity was dose-dependent (maximal action at a 2-mM concentration), appeared after 2 days in culture, and was maximal after 5 days. After sodium butyrate removal from the culture medium, K 562 cells recovered their normal NK susceptibility within 4 days. In cold-target competition experiments, untreated K 562 cells acted as better competitors than butyrate-treated K 562 cells. Target-binding cell assays and specific NK cell removal on K 562 monolayers demonstrated that NK cells were less adherent to butyrate-treated than to control K 562 cells. From these data, it could be postulated that sodium butyrate modulates the expression of some target structure(s) involved in NK target binding. Five subclones of K 562 have been selected for exhibiting distinct, stable properties with regard to their cellular commitment (either to erythroid or to megakaryocytic lineage). The NK sensitivity of these subclones was roughly identical, and in all of them, butyrate treatment induced similar protection against NK-mediated lysis. Thus, the susceptibility to NK lysis and its modulation did not depend upon the predominant lineage present in each clone. (Author abstract) (32 Refs)

34. EXPRESSION OF ERYTHROID DIFFERENTIATION AND TERMINAL CELL DIVISION IN INDUCED FRIEND LEUKEMIA CELLS.

Gambari R, Marks PA, Rifkind RA, Farace M, Fantoni A
Istituto di Biologia Generale, Universita di Roma, Roma, Italy
Acta Biol Med Ger; 40(4/5):511-518 1981

The relationship between cell cycle and globin gene expression was investigated during late differentiation of Friend leukemia cells (FLC) treated with hemin. Cell growth and cell cycle kinetics in the FLC line DS19 were determined by propidium iodide staining and by flow microfluorometry analysis of relative DNA content. Globin gene expression was studied by molecular hybridization of phenol extracted cytoplasmic RNA with mouse globin (3H)-complementary (c)DNA or by cDNA cellulose chromatography of pulse-labeled (3H)RNA purified from the nuclei or cytoplasm of FLC cultured with the inducer hexamethylenebisacetamide (HMBA) or hemin. In FLC treated with HMBA, accumulation and translation of globin messenger (m)RNA and Hb were associated with terminal cell division. Since hemin did not induce expression of genes responsible for terminal cell divisic. .he relationship between cell cycle and globin gene expres..n was determined during late differentiation of FLC treated with hemin. Results demonstrate that terminal cell division does not necessarily occur in cells which accumulate both globin mRNA and Hb, suggesting that these features are not necessarily coupled in leukemia erythroid differentiation. (14 Refs)

35. THE ROLE OF HEME IN THE REGULATION OF IRON UPTAKE DURING FRIEND CELL DIFFERENTIATION.

Hradilek A, Fuchs O, Borova J, Neuwirt J
Dept. Pathological Physiology, Faculty General Medicine, Charles Univ., 128 53 Prague, Czechoslovakia
Acta Biol Med Ger; 40(4/5):519-523 1981

In the Friend erythroleukemia cell (FLC) line 707, iron uptake by hemin was more pronounced after induction of erythroid differentiation by dimethylsulfoxide. No such rise in uptake occurred in the FLC line Fw, which is deficient in ferrochelatase activity and does not synthesize Hb after induction of differentiation by sodium butyrate. Significance of the findings is discussed. (9 Refs)

36. EFFECT OF DEXAMETHASONE ON HEXAMETHYLENE BISACETAMIDE-INDUCED FRIEND CELL ERYTHR ODIFFERENTIATION.

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Cancer Res; 42(2):513-518 1982

Friend erythroleukemic cells can be induced to differentiate by chemicals such as dimethyl sulfoxide and hexamethylene bisacetamide (HMBA) in a dose-dependent manner. Others have shown that dexamethasone and other steroid hormones can inhibit the differentiation induced by dimethyl sulfoxide. We show here that dexamethasone has a dual mode of action on the HMBA-induced differentiation of a Friend cell line, DS19. At concentrations above 10(-10) M, dexamethasone inhibits the

HMBA-induced differentiation in DS19 cells. We further found that, as the concentration of HMBA is reduced, the amount of dexamethasone required to inhibit differentiation increases. Such inhibition seems to act primarily by decreasing the probability that a cell will become committed to differentiate. Besides, dexamethasone does not inhibit hemoglobin synthesis of cells which are committed in its absence. In addition, we show that sensitivity to the inhibition by dexamethasone is inversely related to the inducibility of of cell populations. The second action of dexamethasone, observed at concentrations between 10(-10) and 10(-13) M, is to increase the proportion of hemoglobin-containing cells relative to that for cells cultured in its absence. The degree of this synergistic effect for induced differentiation is inversely related to the level of induction in the absence of dexamethasone and thus is best observed in the cells cultured at low levels of HMBA. We present evidence that this synergistic effect may be due to an increased viability of the induced cells and possibly an increase in the proliferative capacity of these cells. (Author abstract) (19 Refs)

37. ALTERATIONS IN CALCITONIN AND PARATHYROID HORMONE RESPONSIVENESS OF ADENYLATE CYCLASE IN F9 EMBRYONAL CARCINOMA CELL TREATED WITH RETINOIC ACID AND DIBUTYRYL CYCLIC AMP.

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J Cell Physiol; 109(3):453-459 1981

Adenylate cyclase (AC) was assayed by measuring conversion of (alpha-32P) ATP to cyclic (32P) AMP at 37 C in F9 embryonal carcinoma cells induced to differentiate to endodermal cells by exposure to 30 nanog/ml retinoic acid. Basal, guanosine triphosphate-, and fluoride-stimulated AC activities increased as induced change in cells occurred. The endodermal cell type showed little AC stimulation in response to calcitonin, and a strong increase in parathyroid hormone response. This behavior is the opposite of that in untreated F9 cells, which show greatest cyclic AMP stimulation with calcitonin, and little with parathyroid hormone. Treatment of retinoic acid-generated endodermal cells with dibutyryl cyclic AMP yields a type with neural-like morphology. AC in these cells was stimulated by parathyroid hormone, prostaglandin E1, isoproterenol, and epinephrine, but calcitonin response was lost. The possible metabolic role during development for calcitonin and parathyroid hormone indicated by these results implies that changes in AC hormonal responsiveness could serve as markers of early differentiation stages. (25 Refs)

38. DIFFERENTIATION-SPECIFIC INCREASE OF CAMP-DEPENDENT PROTEIN KINASE IN THE 3T3-L1 CELLS.

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Dept. Pharmacology, Harvard Medical Sch., Boston, MA, 02115
J Biol Chem; 257(1):298-306 1982

The specific activity of cAMP-dependent protein kinase was studied during the differentiation of 3T3-L1 fibroblasts into adipocytes. 8-Azido-adenosine 3':5'-monophosphate, a photoaffinity-labeling analogue of cAMP, was used to quantitate the regulatory subunits of the Type I and Type II cAMP-dependent protein kinase in cell extracts. The catalytic subunit of cAMP-dependent protein kinase was studied by assaying for cAMP-dependent histone kinase activity. A significant increase in cAMP-dependent protein kinase activity was observed during the adipose development of 3T3-L1 cells, promoted by 3-isobutyl-1-methylxanthine (IBMX), dexamethasone, and insulin. This increase was attributed primarily to a three- to six-fold increase in the Type I cAMP-dependent protein kinase, as demonstrated either by the incorporation of 8-azido-adenosine 3':5'-monophosphate into the 47,000-dalton regulatory subunit of the Type I kinase or by the separation and analysis of the Type I and Type II kinases through DEAE-Sephacel column chromatography. The time course of increase in the Type I cAMP-dependent protein kinase preceded increases both in malic enzyme and glutamine synthetase activities and was maximal at 5 days after initiation of the hormone treatment. The induction of cAMP-dependent protein kinase does not appear to involve an increase in intracellular cAMP. The addition of either 1 mM dibutyryl cAMP or 0.5 mM IBMX had little or no effect on the expression of cAMP-dependent protein kinase activity in the 3T3-L1 cells, although a marked increase in cAMP phosphodiesterase activity was observed in cells treated with dibutyryl cAMP. The increased expression of the Type I cAMP-dependent protein kinase in 3T3-L1 cells appears to be determined by the operation of the intrinsic differentiation program in these cells; it was observed in both the hormone-induced as well as spontaneously differentiated 3T3-L1 cells but was not observed in a control clone of 3T3, the 3T3-C2 cells, treated with IBMX-dexamethasone and insulin. These results suggest that the 3T3-L1 cells may provide an ideal system for studying the mechanism of regulation of cAMP-dependent protein kinase and its functional significance during development and differentiation. (Author abstract) (45 Refs)

OTHER RELATED STUDIES

39. THE CULTURE OF HUMAN TUMOR CELLS IN SERUM-FREE MEDIUM.

Barnes D, Van der Bosch J, Masui H, Miyazaki K, Sato G
Dept. Biological Sciences, Univ. Pittsburgh, Pittsburgh, PA, 15260
Methods Enzymol; 79:368-391 1981

Methods are described for serum-free growth of established cell lines and primary cultures of several lines of human tumors. The serum-free medium (SFM) HC supported growth of HC84S, a human colon carcinoma line which is transplantable in nude mice. Some, but not all, transplantable human colon tumor lines tested were established in this medium, including a carcinoma taken directly from a patient. Both tumorigenic and nontumorigenic clones of human astrocytoma line HA24 were grown in SFM. A medium LA supported primary cultures of human lung adenocarcinoma T291, transplantable in nude mice. Another medium grew T222, a line of human lung epidermoid carcinoma, transplantable in nude mice, but multipassage serum-free growth could not be maintained for either lung carcinoma line. A SFM supported line MCF-7, derived from a metastatic pleural effusion of human breast cancer. Some, but not all of the breast tumor lines tested, grew in this medium. A factor purified from serum stimulated spreading in these and other cells. A method is described for isolation in SFM of human tumor cells of epithelial origin. In several instances, growth and differentiated properties of cells grown in SFM were better than when grown in serum-supplemented medium, indicating that serum-free media may duplicate more closely the in vivo environment. (28 Refs)

40. EMERGENCE OF HAMSTER FIBROBLAST TUMORS IN NUDE MICE - EVIDENCE FOR IN VITRO SELECTION LEADING TO LOSS OF GROWTH FACTOR REQUIREMENT.

Perez-Rodriguez R, Chambard JC, Van Obberghen-Schilling E, Franchi A, Pouyssegur J
Centre de Biochimie - CNRS, Universite de Nice, Parc Valrose, 06034 Nice, France
J Cell Physiol; 109(3):387-396 1981

Chinese hamster lung fibroblasts (line CC139) obtained from tumors, were established in vitro, and exponentially growing cultures subjected to mutagenesis by a stock of ethyl methane sulfonate (0.3 ul/ml of complete medium) for 16 hr. After 5-6 generations, selection for colony formation in soft agarose was attempted. Tumorigenesis in nude mice and DNA synthesis in serum-free medium were evaluated for 15 anchorage-independent colonies selected. All of the clones but one had lost both the high anchorage and high serum dependence characteristic of the original CC139 fibroblast line. Partial loss of serum growth factor requirement acquired in vivo seems to be an essential malignant character for bypassing the hormonal growth restraints imposed by the host upon the CC139 cells. (30 Refs)

41. FUNCTIONAL PROPERTIES OF CONTINUOUSLY CULTURED HUMAN T LYMPHOCYTES.

Boylston AW, Anderson RL, Haworth CA
Dept. Experimental Pathology, St. Mary's Hosp. Medical Sch., London W2 1PG, England
Clin Exp Immunol; 43(2):329-335 1981

Properties of human T lymphocytes (CCTC) maintained in continuous culture by a new method were studied. CCTC did not proliferate in response to phytohemagglutinin, concanavalin A, or allogeneic lymphocytes. CCTC inhibited proliferation of normal cells in mixed lymphocyte culture and were cytotoxic to a number of human lymphoid cell lines. Properties of CCTC differed significantly from those of cells grown on T-cell growth factor. (16 Refs)

42. ASSAY OF GROWTH INHIBITION LYMPHOBLASTOID CELL CULTURES.

Evinger M, Pestka S
Columbia Univ. Medical Center, 630 West 168th St., New York, NY, 10032
Methods Enzymol; 79:362-368 1981

An assay is described for the measurement of interferon (IF) antiproliferative activity with the human lymphoblastoid Daudi cell line, originally obtained from a patient with Burkitt's lymphoma. Suspension cultures were maintained at 36 +-0.5 C in RPMI-1640 medium, with 10% calf serum and 50 ug/ml of gentamicin. After centrifugation cells were dispersed and 1 ml of suspension plated in each 16-mm well of Linbro culture plates. IF preparations were diluted so that 5-30 units IF were added to each well, and the preparations incubated at 36.5 C for 3 days. While cell proliferation may be measured by either counting cells with a Coulter counter or by measuring incorporation of labeled precursors into macromolecules (eg, 3H-thymidine into DNA), the simpler method of counting cells was employed. Both human leukocyte IF and fibroblast IF were used; results are given as percentage of control growth. For leukocyte IF, concentrations of 3, 6, 15, 30, and 60 units/ml resulted in 47, 39, 31, 17.5 and 10% of the growth in control cells. With fibroblast IF, concentrations of 10, 25, 50, 100, and 200 units/ml resulted in 78, 69, 48, 35, and 9% of the growth in control cells. The use of Daudi cells is reported to be one of the most sensitive methods for measuring IF antiproliferative ability. (9 Refs)

43. CORRELATION BETWEEN THE LOSS OF THE TRANSFORMED PHENOTYPE AND AN INCREASE IN SUPEROXIDE DISMUTASE ACTIVITY IN A REVERTANT SUBCLONE OF SARCOMA VIRUS-INFECTED MAMMALIAN CELLS.

Fernandez-Pol JA, Hamilton PD, Klos DJ
Nuclear Medicine Lab., Veterans Admin. Medical Center, St. Louis, MO, 63125
Cancer Res; 42(2):609-617 1982

We have studied the effects of paraquat (methyl viologen), a herbicide that increases intracellular production of superoxide radical, on the viability of virus-transformed and nontransformed normal rat kidney (NRK) cells in culture. We have shown that a low concentration of paraquat (12.5 uM) is cytotoxic toward virus-transformed cell lines, including Kirsten sarcoma virus- and SV40-transfor med NRK cells. The corresponding untransformed NRK cells were resistant to the same and a four-fold higher concentration of paraquat. There was good correlation between the susceptibility of transformed and untransformed cells to paraquat cytotoxicity and their ability to increase the superoxide dismutase (SOD) enzymatic activity. We found that paraquat is cytotoxic toward Kirsten sarcoma virus-transformed and SV40-transformed NRK cells which showed low intracellular SOD activity. The relationship between SOD activity and paraquat cytotoxicity was strengthened by the finding that the tolerance of NRK cells to the drug was associated with high intracellular SOD activity. This report also describes the isolation of a revertant (revertant RE8G3) cell line derived from Kirsten sarcoma virus-transformed NRK cells after paraquat treatment which contains SOD activity at levels much higher than those found in NRK cells. This revertant is undistinguishable from NRK cells with respect to its lack of transformed cell properties. Not only are these cells normal morphologically but also they do not grow in soft agar, an in vitro property that closely correlates with in vivo tumorigenicity. Several biological and biochemical properties of RE8G3 cells, including growth characteristics, surface receptors for both transferrin and epidermal growth factor (EGF), and the EGF-dependent 32P phosphorylation of specific membrane polypeptides have been studied. The most interesting conclusion that can be drawn from these studies is that there is a correlation between loss of the transformed phenotype and an increase in both *EGF receptors* and EGF-dependent 32P phosphorylation of a mol wt 170,000 membrane-associated protein. (Author abstract) (31 Refs)

44. BESTATIN, A STIMULATOR OF POLYSOME ASSEMBLY IN T CELL LYMPHOMA (L 5178Y).

Muller WE, Zahn RK, Maidhof A, Schroder HC, Umezawa H
Institut fur Physiologische Chemie, Universitat Mainz, Duesbergweg, 6500 Mainz, W. Germany
Biochem Pharmacol; 30(24):3375-3377 1981

The aminopeptidase B inhibitor bestatin (BES) was tested for its effects on proliferation of exponentially growing L 5178y lymphoma cells and on polysome (PS) assembly in nonproliferating cells. No reduction of cell proliferation occurred in cultures incubated with BES (1-100 ug/ml) for 72 hr. PS and monosomes were isolated by sucrose gradient centrifugation from L 5178y cells after a 2-hr incubation with BES. Relative amounts (Optical Density units) of monosomal and polysomal areas in control cells were 1.54 and 1.75, respectively. BES (1-50 ug/ml) caused an a dose-dependent increase in PS formation, with max stimulation occurring at 10 ug/ml (polysomal area, 2.65; monosomal area, 0.67). Concentrations greater than 50 ug/ml did not influence PS assembly. The possible role of

BES in regulation of immune responses is discusse d briefly. (18 Refs)

45. PATTERNS OF PROLIFERATION AND DIFFERENTIATION OF IRRADIATED HAEMOPOIETIC STEM CELLS CULTURED ON NORMAL 'STROMAL' CELL COLONIES IN VITRO.

Mori KJ
Dept. Microbiology, Faculty Medicine, Kyoto Univ., Kyoto 606, Japan
J Radiat Res (Tokyo); 22(3):344-351 1981

Irradiated bone marrow cells were overlaid on normal, unirradiated 'stromal' cell colonies and the pattern of proliferation and differentiation of hemopoietic stem cells was followed in vitro. Eight-wk-old male DDY mice were used for bone marrow cell culture, and 10-12-wk-old mice of the same strain were used as recipients for the assay of hemopoietic stem cells (CFUs). Culture of the bone marrow cells resulted in formation of adherent cell colonies within 3 wk. On these colonies were overlaid bone marrow cells irradiated with various doses of x-rays. Results show that the number of CFUs was reduced only to 48% of the control value even after 300 R. The normal level was attained within 2 wk of irradiation. A slight increase in the number of granulocyte-macrophage percursor cells (CFUc) in the late period was noted in cultured 300 R-irradiated bone marrow cells. Proliferation of hemopoietic stem cells was supported, with the degree of stimulation depending on the starting cellular concentration. No significant changes were observed in the concentration of either CFUs or CFUc regardless of the dose of irradiation to overlaid marrow cells. A dose-dependent decrease in CFUs and CFUc was observed within the culture in which both stem cells and stromal cells were simultaneously irradiated. The balance of self-replication and differentiation of the hemopoietic stem cell may be affected only when the hemopoietic microenvironment is perturbed. (16 Refs)

46. CAMP RECEPTOR PROTEINS AND PROTEIN KINASES IN HUMAN LYMPHOCYTES: FUNDAMENTAL ALTERATIONS IN CHRONIC LYMPHOCYTIC LEUKEMIA CELLS.

Weber W, Schwoch G, Wieickens K, Gartemann A, Hilz H
Physiologisch-Chemisches Institut, der Universitat Hamburg, Universitats-Krankenhaus Eppendorf, Martinistrabe 52, D-2000 Hamburg 20, W. Germany
Eur J Biochem; 120(3):585-592 1981

To study the significance of cAMP receptor proteins and protein kinase activities in tumor dys-differentiation, peripheral blood lymphocytes from normal donors and from patients with chronic lymphocytic leukemia were isolated by Percoll gradient centrifugation, and analyzed. Both normal and tumor lymphocyte populations were devoid of cell proliferation. Normal human lymphocytes contained about equal activities of histone kinase and 'casein kinase'. The tumor lymphocytes, however, exhibited drastically reduced values of all three protein kinase activities, the most pronounced decrease being observed with the 'casein kinase'. Total high-affinity cAMP-binding sites (= regulatory subunits R of cAMP-dependent protein kinases Type I and II) in leukemic lymphocytes were also strongly reduced. The findings in lymphocytes of chronic lymphocytic leukemia cannot be explained by the fact that they are B-type lymphocytes. Control experiments revealed that normal B cells do not differ significantly from total blood lymphocytes with respect to the parameters mentioned above. Biochemical correlates of the dys-differentiated status of these non-proliferating cells from patients with chronic lymphocytic leukemia, then, are represented by reduced levels of protein kinases, of basal cAMP as well as by changes in the R-protein pattern. The data indicate that both protein kinases Type I and Type II may relate to the state of differentiation, independently from cell proliferation. (57 Refs)

47. PROTEIN INDUCTION PROCESS MAY POSSIBLY EXPLAIN KINETICS OF COMMITMENT OF MURINE ERYTHROLEUKEMIA CELLS TO DIFFERENTIATION.

Svetina S
Inst. Biophysics, Medical Faculty, Edvard Kardelj Univ., Ljubljana, Yugoslavia
Acta Biol Med Ger; 40(4):525-526 1981

A model of commitment kinetics for elucidation of molecular mechanisms underlying the action of inducers of murine erythroleukemia cell differentiation is presented. The model may explain why such differentiation can be stimulated by inducers which act on different sites. The model can be applied to the rate of accumulation of protein, which can be altered by changing a molecular parameter at any of several levels. (5 Refs)

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CELL AND SERUM DERIVED GROWTH EFFECTORS

1. ISOLATION FROM MURINE SARCOMA CELLS OF NOVEL TRANSFORMING GROWTH FACTORS POTENTIATED BY EGF.

Roberts AB, Anzano MA, Lamb LC, Smith JM, Frolik CA, Marquardt H, Todaro GJ, Sporn MB
Lab. Chemoprevention, NCI, NIH, Bethesda, MD, 20205
Nature; 295(5848):417-419 1982

Two distinct classes of intracellular transforming growth factors (TGF) were isolated from Moloney sarcoma virus (MSV)-transformed 3T3 cells. One TGF competed with epidermal

growth factor (EGF) for receptor binding sites, whereas the other did not. After high-performance liquid chromatography purification, the colony-forming activity of the TGF that do not compete with EGF for receptor bin ding was enhanced 100-fold by the addition of EGF. The abiliity to enhance growth-stimulating effects of TGF was specific for EGF, as insulin, the insulin-like growth factors, platelet-derived growth factor, and nerve growth factor did not show this property. In contrast, the colony-forming activity of TGF that compete with EGF for receptor binding was not potentiated by EGF. The role of the new TGF in malignant transformation, wound repair, and embryogenesis are discussed. (24 Refs)

2. LONG-TERM EPIDERMAL GROWTH FACTOR-RECEPTOR INTERNALIZATION AND PROCESSING IN QUIESCENT HUMAN FIBROBLASTS.

King AC, Cuatrecasas P
Dept. Molecular Biology, Wellcome Res. Lab., Research Triangle Park, NC, 27709
J Supramol Struct Cell Biochem; 17(4):377-387 1981

Long-term internalization and processing of epidermal growth factor (EGF) was studied in quiescent human foreskin fibroblasts (HF). HF were exposed in vitro to unlabeled or 125I-labeled EGF. Pretreatment of HF with 5 nanog (ng) EGF for 2 hr followed by removal of unbound EGF produced no potentiation of DNA synthesis. When EGF was added back to cultures for 14 hr, DNA synthesis was identical to that produced by continuous EGF exposure, suggesting that clearance of cell surface receptors by treatment with EGF does not reduce cellular response to EGF at the concentrations tested. Addition of methylamine (MeNH2), which prevents lysosomal degradation of EGF-recptor complexes, produced an increase in cell-associated EGF compared with controls. This increase was the result of accumulation of EGF within the cells. Cells were apparently able to internalize 125I-EGF even after severe reduction of surface receptors. Max uptake of 125I-EGF in the presence of MeNH2 was 0.27 femtomoles (fmol)/min/culture for untreated cells and 0.019 fmol/min/culture for cells after clearance of surface receptors by pretreatment with EGF. Mitogenic response of fibroblasts to EGF was not reduced by down regulation of surface receptors. (24 Refs)

3. ALTERATION OF EPIDERMAL GROWTH FACTOR-DEPENDENT PHOSPHORYLATION DURING RAT LIVER REGENERATION.

Rubin RA, O'Keefe EJ, Earp HS
Cancer Cell Biology Pogram, Cancer Res. Center, Univ. North Carolina, Chapel Hill, NC, 27514
Proc Natl Acad Sci USA; 79(3):776-780 1982

Epidermal growth factor (EGF) stimulates membrane protein phosphorylation in a human cell line, A-431. The known hepatic mitogenic action of EGF and the reduction in EGF receptor number that occurs during liver regeneration led us to study whether EGF-dependent protein kinase activity was present in rat liver and whether its activity was altered after partial hepatectomy. Liver membranes preincubated with or without EGF, were phosphorylated (0 C, 15 sec) and subjected to NaDodSO4/polyacrylamide gel electrophoresis and autoradiography. In microsomal fractions, EGF at 5-2,000 ng/ml produced a dose-r elated stimulation of 32P incorporation into a single 170,000-dalton protein (p170). In plasma membranes, a similar EGF-dependent phosphorylation was present and was substantially enriched relative to the microsomal fraction. Acid hydrolysis of labeled microsomal fraction followed by phosphoamino acid determination revealed that EGF stimulated 32P incorporation into phosphotyrosine residues. The EGF-dependent phosphorylation of p170 was compared in microsomal fractions isolated from rats 36 hr after partial hepatectomy or sham operation. In the absence of EGF, in vitro labeling of p170 was similar. EGF stimulated the labeling of p170 in both groups, but the response was clearly diminished after partial hepatectomy. In the presence of EGF, the labeling of p170 in microsomal fraction from regenerating livers was only 47 +- 6% of that observed in membranes from sham-operated rats (p less than 0.005). Reduction of EGF-dependent phosphorylation during liver regeneration paralleled the loss of binding of 125I-labeled EGf. An increase in the EGF-dependent phosphorylation of a 130,000-dalton protein was also observed after partial hepatectomy. The increase in the amount of this phosphoprotein was roughly equal to the loss of EGF-stimulated p170 phosphorylation. Several additional proteins showed increased phosphorylation in membranes from partially hepatectomized rats. These findings indicate that alterations in membrane tyrosine residue phosphorylation occur during regulated growth in vivo. (Author abstract) (28 Refs)

4. MICROTUBULES AND MICROFILAMENTS IN FIXED AND PERMEABILIZED CELLS ARE SELECTIVELY DECORATED BY NERVE GROWTH FACTOR.

Nasi S, Cirillo D, Naldini L, Marchisio PC, Calissano P
Centro degli Acidi Nucleici, Consiglio Nazionale delle Ricerche, Via Romagnosi 18A, 001296 Roma, Italy
Proc Natl Acad Sci USA; 79(3):820-824 1982

A specific antibody against nerve growth factor (NGF) and indirect immunofluorescence microscopy have been used to follow the in vitro binding of NGF to cells made permeable to large molecules. All cells tested, both target (sensory neurons and PCI2 cells) and nontarget (3T3, BKH 2I, C6 glioma cells), revealed a decoration of cytoskeletal structures which on the basis of their form, reactivity with antibodies, and sensitivity to specific drugs may be identified as microtubules (MTs) and microfilaments (MFs). The decoration of either structure depends on the fixation and permeabilization conditions: MFs, in the form of stress fibers, are stained by NGF when the plasma membrane is permeabilized with methanol/acetone; MTs become intensely stained when the plasma membrane is solubilized with a nonionic detergent in the presence of a MT-stabilizing medium. The two procedures do not affect the staining of these structures with specific antibodies. Binding of 125I-labeled NGF to PCI2 cells was not competitively inhibited by a 100-fold exc ess of several positively charged proteins but it was markedly decreased in the presence of DNase I. 125I-Labeled NGF interacted with MTs and F-actin (fixed with paraformaldehyde) in a range of concentrations similar to that used for their cellular localization with NGF-anti-NGF. Our studies show that the specificity and affinity of NGF binding to MTs and MFs is in the range of that of antibodies against tubulin and actin. The possible relevance of these findings to the mechanism of action of NGF in target cells is discussed. (Author abstract) (27 Refs)

5. MAMMALIAN CELL PROLIFERATION IS REGULATED BY THE SYNERGISTIC ACTIONS OF MULTIPLE GROWTH FACTORS.

Pledger WJ, Hart CA, Wharton WR
Dept. Pharmacology and Cancer Res. Center, Univ. North Carolina Medical Sch., Chapel Hill, NC, 27514
Adv Exp Med Biol; 138:287-300 1982

Regulatory events in the G1 phase of the mammalian cell cycle, regulation by serum factors and the process by which the transformation alters the normal regulation were studied. Mechanisms of action can be investigated after the growth factors and their ordered requirement in cell cycle traverse is learned. Comparison of the normal cells to transformed cells can lead to the understanding of how transformed or neoplastic cells override growth dependency on mitogenic signals. (18 Refs)

6. STIMULATION OF TYROSINE-SPECIFIC PHOSPHORYLATION BY PLATELET-DERIVED GROWTH FACTOR.

Ek B, Westermark B, Wasteson A, Heldin CH
Biomedical Center, Inst. of Medical and Physiological Chemistry, Uppsala Univ., Uppsala, Sweden
Nature; 295(5848):419-420 1982

The ability of platelet-derived growth factor (PDGF) binding to cellular receptors to stimulate kinase activity was studied in membranes prepared from cultured human foreskin fibroblasts or glial cells. PDGF stimulated the incorporation of 32P into membrane proteins of both fibroblasts and glial cells, promoting, in particular, the phosphorylations of a 175,000-molecular wt (Mr) and a 130,000-Mr protein, in comparison with the control incubation, in which no growth factor was added. Slight effects of PDGF were also seen in other components of the autoradiogram, associated with both increased and decreased phosphorylation. In the presence of epidermal growth factor (EGF), 32P incorporation increased preferentially in a 170,000-Mr protein of the fibroblast membrane preparation and in both a 170,000-and a 150,000-Mr component of the glial cell membrane preparation. The results indicated that different proteins serve as substrates for the kinases stimulated by the two factors. In membranes of the A431 epithelial cell line, only EGF appreciably increased phosphorylation. The specificity of the PDGF-stimulated kinase activity was studied in relation to the type of phospho-accepting amino residue involved. The mechanisms of action of EGF and PDGF are discussed. (34 Refs)

7. TWO-FACTOR REQUIREMENT FOR MURINE MEGAKARYOCYTE COLONY FORMATION.

Williams N, Eger RR, Jackson HM, Nelson DJ
Dept. Physiology, Melbourne Univ., Victoria 3052, Australia
J Cell Physiol; 110(1):101-104 1982

A synergistic requirement for both megakaryocyte colony-stimulating factor (MK-CSF) and megakaryocyte potentiator (MKP) in growth of C57BL/6 mouse bone marrow-derived megakaryocytes was demonstrated, based on gel filtration of murine myelomonocytic leukemia cell line (WEHI-3)-conditioned medium. Results implicated two factors when either 20% fetal bovine serum-supplemented or serum-free McCoy's medium was used in the megakaryocyte progenitor cell assay. The MKP has an apparent mol wt of 100,000 daltons, whereas the mol wt range of MK-CSF is about 20,000-65,000 daltons. Small but detectable numbers of megakaryocyte colonies were found when fractions separated on Sephadex G-150 and supposedly containing only MK-CSF were used for potentiation, suggesting that MKP might not be needed or that separation of the two factors was not total. No colony formation above background level was observed in experiments with serum-free medium unless both factors were present. In situ scoring of acetylcholinesterase-positive colonies demonstrated that the factors act prior to the detection of enzyme in the bioassay, but such experiments could not establish that both factors are not necessary because small immature megakaryocytes contain small amounts of acetylcholinesterase and would not be detected with the procedure used. (23 Refs)

8. GROWTH OF HUMAN FORESKIN FIBROBLASTS IN A SERUM-FREE DEFINED MEDIUM WITHOUT PLATELET-DERIVED GROWTH FACTOR.

Weinstein R, Hoover GA, Majure J, Van der Spek J, Stemerman MB, Maciag T
Dept. Medicine, Harvard Medical Sch., Boston, MA, 02215
J Cell Physiol; 110(1):23-28 1982

Neonatal human foreskin fibroblasts (NHFF) were grown in a defined medium consisting of epidermal growth factor (100 nanogram (ng)/ml), insulin (100 ng/ml), transferrin (10 ug/ml), thrombin (1 ug/ml), ascorbic acid (10 ug/ml), and hydrocortisone (5 x 10(-5) M) in a 1:1 mixture of Dulbecco's modified Eagle's medium and Ham's F-12 supplemented with ovalbumin (1 mg/ml) and trace elements. Growth of NHFF in this defined medium was comparable to that with 5% fetal bovine serum. In the defined medium, insulin, transferrin, thrombin, and hydrocortisone were necessary for growth, but platelet-derived growth factor, fibroblast growth factor, and somatomedin (from human plasma Cohn fraction IV) were not. (63 Refs)

9. DEMONSTRATION OF COMPETENCE AND PROGRESSION ACTIVITIES FOR HUMAN FIBROBLASTS.

Bright MD, Gaffney EV
Microbiology Program, Pennsylvania State Univ., University Park, PA, 16802
Exp Cell Res; 137(2):309-316 1982

Human embryonic lung fibroblasts (HEL) completed 4.5 population doublings in 6 days when maintained in DMEM supplemented with 10% human whole blood serum (WBS), plasma-derived serum (PDS) or defibrinogenated plasma containing 10 mM CaCl2. Plasma in the absence of additional calcium promoted less growth. Sera and plasma chromatographed through carboxymethyl Sephadex (CMS) supported only one population doubling. Increased growth resulting in three doublings was observed in CMS-treated WBS or PDS supplemented with commercially prepared platelet-derived growth factor (PDGF), The magnitude of this PDGF response was dependent on serum concentration. A significant increase in the proportion of cells incorporating [3H]thymidine was observed in confluent cultures exposed to PDGF prior to incubation in WBS-CMS or PDS-CMS indicating competence and progression activities for human fibroblasts. In contrast, cells maintained in the presence of plasma-CMS failed to grow in response to PDGF. Factors bound to CMS columns restored growth-promoting activity to PDGF-supplemented WBS-CMS, PDS-CMS and plasma-CMS. However, growth-promoting CMS-bound components from plasma were lost during dialysis through membranes excluding materials above 12,000 MW. (Author abstract) (20 Refs)

10. TRANSFORMING GROWTH FACTOR(S) PRODUCTION ENABLES CELLS TO GROW IN THE ABSENCE OF SERUM: AN AUTOCRINE SYSTEM.

Kaplan PL, Anderson M, Ozanne B
Dept. Microbiology, Univ. Texas Health Science Center, 5323 Harry Hines Blvd., Dallas, TX, 75235
Proc Natl Acad Sci USA; 79(2):485-489 1982

Kirsten murine sarcoma virus (KiMSV)-transformed rat-1, normal rat kidney (NRK), and BALB/c 3T3 cells are capable of continual growth in a serum-free medium supplemented with transferrin and insulin but with no exogenous mitogenic growth factors. Cells transformed by a mutant of KiMSV that is temperature sensitive for the maintenance of transformation grow in this medium at the permissive temperature only. At the nonpermissive temperature, growth is dependent upon the presence of serum-free conditioned medium from the transformed cells. Normal rat-1 cells are also dependent upon factors from the transformed cells for growth in this serum-free/mitogen-free medium. The serum-derived growth factors, epidermal growth factor, and fibroblast growth factor have no effect on the transformed cells, although epidermal growth factor can replace transforming growth factors produced by KiMSV-transformed cells for the growth of rat-1 cells. Growth of the transformed cells in serum-free medium at clonal densities is dependent upon the presence of conditioned medium collected from the same cells grown to high densities. These results show that (i) growth in serum-free/mitogen-free medium is a general property of KiMSV-transformed cells and (ii) growth of the transformed cells in this medium is dependent upon the presence of growth factor s known to be produced by the cells, and they provide support for the hypothesis that serum-free growth of KiMSV-transformed cells is dependent upon ectopically produced growth factors. (Author abstract) (19 Refs)

11. FACTORS INVOLVED IN THE CONTROL OF PROLIFERATION OF BOVINE CORNEAL ENDOTHELIAL CELLS MAINTAINED IN SERUM-FREE MEDIUM.

Giguere L, Cheng J, Gospodarowicz D
(c/o Gospodarowicz) Cancer Res. Inst., Univ. California Medical Center, San Francisco, CA, 94143
J Cell Physiol; 110(1):72-80 1982

Conditions for growth of bovine corneal epithelial (BCE) extract cell cultures on BCE cell-produced extracellular matrix-coated dishes in the absence of serum are described. Cells proliferate actively in a medium supplemented with human high-density lipoprotein, transferrin, insulin, and bovine brain fibroblast growth factor or epidermal growth factor. Subcultures of over 80 generations have been obtained on the medium. The medium permits comparison of serum-free vs serum-containing media and may also lead to a system closer to the in vivo environment of the corneal epithelium in which protein concentrations are much lower than in plasma. (25 Refs)

12. ABILITY OF ENDOTHELIAL CELLS TO CONDITION CULTURE MEDIUM.

Gajdusek CM, Schwartz SM
Dept. Pathology, Univ. Washington, Seattle, WA, 98195
J Cell Physiol; 110(1):35-42 1982

Bovine aortic endothelial (BAE) cells, incubated as monolayers for 48 hr in serum-free medium, were shown to produce a factor that permitted growth of BAE cells in serum-limited (0.2% plasma-derived serum, PDS) medium that was comparable to growth normally obtained with 5% PDS. The factor dialyzed through tubing with a 6,000-8,000 dalton limit, and did not stimulate quiescent 3T3 cells, bovine aortic smooth muscle cells, or human neonatal skin fibroblasts as did a second previously-described polypeptide in BAE cell-conditioned medium that is retained by dialysis tubing. A similar conditioned effect was noted in medium conditioned by exposure to smooth muscle cells and human dermal fibroblasts, suggesting that the conditioning effect is not limited to BAE cells. (40 Refs)

13. SPINAL CORD NEURONOTROPHIC FACTORS (SCNTFS): I. BIOASSAY OF SCHWANNOMA AND OTHER CONDITIONED MEDIA.

Longo FM, Manthorpe M, Varon S
Dept. Biology, Univ. California, San Diego, La Jolla, CA, 92093
Dev Brain Res; 3(2):277-294 1982

Trophic- and neurite-promoting factors directed to spinal cord neurons were investigated. Culture behavior of neurons dissociated from 4-day old chick embryo lumbar spinal cord (LC4) is described. Seeding density, media, and substrata were selected to minimize the number of neurons that would survive without appropriate supplements, thus maximizing the opportunity to detect and analyze sources of exogenous factors. Conditioned media from Schwann and muscle cells contained both survival and neurite-promoting factors for LC4 neurons. Examination of Schwannoma-conditioned medium has revealed that the two LC4 cell activities can be physically separated. (69 Refs)

14. LARGE-SCALE PREPARATION AND CHARACTERIZATION OF HUMAN COLONY-STIMULATING FACTOR.

Okabe T, Nomura H, Sato N, Ohsawa N
Third Dept. Internal Medicine, Univ. Tokyo, Hongo-Tokyo, Japan
J Cell Physiol; 110(1):43-49 1982

Methods were developed for cultivation of the T3M-1 human granulocytic colony-stimulating factor (G-CSF)-secreting cell line in roller bottles. Spent medium of the culture (F-10 synthetic + 10% fetal bovine serum) produced very high G-CSF activity in human bone marrow cells. Yield from 45 bottles (570 mm) was 100 liter of cultured medium/mo, which stimulated about 150 million granulocytic colonies of human bone marrow cells. Enzymatic, heat, and pH inactivation tests were performed on the G-CSF, which was shown to contain fractions of 30,000 mol wt (high activity) and 15,000 mol wt (higher activity). (22 Refs)

15. PRELIMINARY CHEMICAL AND BIOLOGIC CHARACTERIZATION OF (FC)TRF: AN FC FRAGMENT-INDUCED T CELL-REPLACING FACTOR.

Thoman ML, Weigle WO
Dept. Immunopathology, Scripps Clinic and Res. Foundation, La Jolla, CA, 92037
J Immunol; 128(2):590-594 1982

Fc fragment stimulation of Lyt-1+2-T lymphocytes triggers release of a T cell-replacing factor, here called (Fc)TRF. Chemical and biologic characterization of (Fc)TRF has been undertaken to provide information for its comparison with other T cell factors. Preliminary chemical characterization by column chromatography and isoelectric focusing suggest (Fc)TRF activity resides in a class of acidic molecules that fall into two size classes of 58-60,000 daltons and 35-40,000 daltons.

This factor is distinct from interleukin 2 (IL 2) as it has no T cell growth-promoting or costimulator activity, although it can also be generated by Con A activation of murine spleen cells. By these results (Fc)TRF has been defined as a unique T cell-replacing activity that promotes differentiation of B lymphocytes to antibody-secreting cells. (Author abstract) (25 Refs)

16. HELPER FACTOR PRODUCTION IN MURINE SECONDARY SYNGENEIC MIXED LEUKOCYTE REACTIONS.

Zuberi RI, Altman A
Dept. Molecular Immunology, Scripps Clinic and Res. Foundation, La Jolla, CA, 92037
J Immunol; 128(2):817-822 1982

Culture supernatants of murine thymocytes or spleen cells responding in a secondary syngeneic mixed leukocyte reaction (SMLR) were studied for their biologic effects on cell-mediated immune responses in vitro. Such supernatants contained helper factor(s) that facilitated the development of alloantigen-specific cytotoxic T lymphocyte (CTL) responses from thymocyte precursors. Thymocytes, but not spleen cells, required activation by allogeneic effect factor (AEF) in primary culture in order to proliferate and produce biologically active mediator(s) during a secondary SMLR. The same culture supernatants possessed, in some instances, weak T cell growth factor (TCGF; IL 2) activity. However, TCGF activity could be dissociated from helper factor(s) active in the CTL induction assay because some culture supernatants that had potent helper activity were devoid of TCGF activity. This lack of TCGF activity was not due to a lower degree of sensitivity of the TCGF assay or to the presence of a selective TCGF inhibitor in the SMLR-derived supernatants, indicating that the helper factor(s) studied is distinct from TCGF. Production of immunoregulatory lymphokines during the SMLR may serve as a physiologically relevant model for studying the role of T cell-derived lymphokines in immunoregulation. (Author abstract) (41 Refs)

17. T CELLS PRODUCE TRF IN RESPONSE TO CON A AND FACTORS IN T CELL HYBRIDOMA SUPERNATANTS.

Raulet DH, Hunig T, Parker DC
Dept. Pathology, Univ. Pennsylvania Sch. Medicine, Philadelphia, PA, 19104
J Immunol; 128(2):908-912 1982

In recent experiments, a soluble factor (TRF) that mediates the differentiation of anti-immunoglobulin (Ig)-activated B cells to Ig-secreting cells has been identified. TRF works in concert with a growth factor, probably IL 2, in the induction of activated B cells. In previous studies, TRF was identified in culture supernatants of activated T cells and accessory cells, and thus the cellular source (T cell or accessory cell) of the factor was not determined. In the present studies, we succeeded in inducing the production of TRF by T cell populations from which accessory cells had been vigorously depleted. Lymph node cells were depleted of accessory cells by nylon wool adherence and anti-Ia and complement treatment; these cells were activated with Con A and a T cell hybridoma supernatant that contains IL 2. Supernatants from these activated T cell cultures supported the differentiation of anti-Ig-activated B cells to Ig secreting cells. These results show that T cells produce the differentiation factor, and further that they do so in response to ligand (Con A) plus a T cell-derived factor. (Author abstract) (29 Refs)

18. LYMPHOKINES: THEIR ROLE IN LYMPHOCYTE RESPONSES. PROPERTIES OF INTERLEUKIN 1.

Oppenheim JJ, Stadler BM, Siraganian RP, Mage M, Mathieson B
Lab. Microbiology and Immunology, Natl. Inst. Dental Res., NCI, NIH, Bethesda, MD, 20205
Fed Proc; 41(2):257-262 1982

Interleukin 1, or IL 1 otherwise known as lymphocyte-activating factor, is a macrophage-derived 12,000-to 15,000-dalton polypeptide. Isoelectric focusing of human IL 1 reveals three peaks at pI's of 5.2, 6.0, and 6.9, respectively. IL 1 can be depleted of lymphocyte-derived IL 2 by SP-Sephade x chromatography. IL 1 augments the mitogenic response of PNA- Lyt 1+ thymocytes, and promotes thymocyte helper functions and B cell antibody production. IL 1 induces stable E rosette formation and the production of lymphokines such as T cell growth factor (IL 2) by peripheral T lymphocytes. Others have shown that IL 1 or closely related factors also stimulate hypothalamic cells to induce fever; induce in vitro fibroblast growth, prostaglandin, and collagenase production; and stimulate hepatocytes to produce acute phase proteins such as serum amyloid A. Murine epidermal cells also produce a 15,000-dalton factor that is mitogenic for thymocytes and may be similar to IL 1. We have recently hybridized spleen cells from mice sensitized with partially purified human IL 1 with a myeloma cell line. Clones have been isolated that produce supernatants that partially inhibit the thymocyte proliferative response to IL 1 but not the T cell growth factor activity of IL 2. Should these hybridoma products prove to be monoclonal anti-IL 1 antibodies, they will facilitate the further purification and characterization of IL 1. (Author abstract) (43 Refs)

19. ERYTHROID PROGENITORS IN POLYCYTHEMIA VERA: DEMONSTRATION OF THEIR HYPERSENSITIVITY TO ERYTHROPOIETIN USING SERUM CULTURES.

Casadevall N, Vainchenker W, Lacombe C, Vinci G, Chapman J, Breton-Gorius J, Varet B
Batiment G. Roussy, CHU Cochin, 75674 Paris Cedex 14, France
Blood; 59(2):447-451 1982

In order to demonstrate whether 'spontaneous' erythroid colonies observed in vitro in polycythemia vera (PV) using standard colony assays were independent from erythropoietin (epo) or exquisitely sensitive to the hormone, we used methyl cellulose serum-free cultures, in which serum was completely replaced by iron-saturated transferrin, alpha-thioglycerol albumin, and low density lipoproteins. Connaught Step III epo was used. In six cases of PV, no epo-independent colony (CFU-E or BFU-E derived) was observed in serum-free conditions, while spontaneous colonies were present after plating the same PV bone marrow in culture with serum. The epo dose-response curves showed a 10 fold increase in the sensitivity to epo of PV erythroid progenitors compared to normal controls. In PV, the first CFU-E and BFU-E colonies were observed after addition of 0.001-0.01 IU/ml of epo, while in controls they appeared at an epo concentration between 0.01 and 0.1 IU/ml. Numbers of spontaneous colonies in cultures with serum compared with the epo dose-response curve in the same patient in serum-free cultures are much higher than expected from the small amount of epo present in the serum. These results confirm that PV erythroid progenitors able to differentiate spontaneously in standard culture conditions are in fact dependent of epo and hypersensitive to the hormone. They show that these abnormal progenitors do not represent a homogeneous population, but exhibit different degrees in their hypersensitivity to epo. Since the small amount of epo present in the

normal serum cannot explain the growth of spontaneous colonies by itself, a hypersensitivity to other serum factors cannot be excluded. (Author abstract) (18 Refs)

20. HUMAN LEUKEMIA CELL LINE K562 RESPONDS TO ERYTHROID-POTENTIATING ACTIVITY.

Gauwerky CE, Lusis AJ, Golde DW
Div. Hematology-Oncology, Dept. Medicine, Univ. California Los Angeles Sch. Medicine, Los Angeles, CA, 90024
Blood; 59(2):300-305 1982

We report that erythroid-potentiating activity (EPA), known to stimulate the proliferation of normal human erythroid precursors in vitro, has growth-promoting effect on human K562 erythroleukemia cells and Friend mouse erythroleukemia cells. Detailed studies were carried out using an EPA produced by a human T-lymphoblast line (Mo). Although EPA has not been purified to homogeneity, several observations indicate that the factor elaborated by Mo cells that stimulates erythroleukemia growth factor is the EPA molecule. The erythroleukemia growth factor cofractionates with EPA using gel exclusion chromatography, isoelectric focusing, and ion exchange chromatography. In addition, the activities exhibit similar kinetics of heat inactivation. A granulocyte-macrophage colony-stimulating factor also elaborated by Mo cells had no effect on growth of the erythroleukemia cells. Other sources of EPA, such as peripheral blood leukocyte-conditioned medium, preparations from urine of anemic patients, and medium conditioned by a human monocyte-like cell line, stimulated erythroleukemia cell growth. Mouse sources of EPA (termed 'burst-promoting activity') stimulated mouse but not human erythroleukemia cells. The availability of cell lines apparently responsive to EPA should prove useful for examining the mode of action of this regulator of erythropoiesis. (Author abstract) (28 Refs)

21. STIMULATION BY INTERFERON OF INDUCTION OF DIFFERENTIATION OF HUMAN PROMYELOCYTIC LEUKEMIA CELLS.

Tomida M, Yamamoto Y, Hozumi M
Dept. Chemotherapy, Saitama Cancer Center Res. Inst., Ina-machi, Kitaadachi-gun, Saitama-ken 362, Japan
Biochem Biophys Res Commun; 104(1):30-37 1982

The effects of purified human leukocyte interferon-alpha (IFA) and partially purified fibroblast interferon-beta (IFB) on induction of differentiation of human promyelocytic leukemia cells (HL-60) into granulocytes and macrophages were studied. HL-60 cells maintained for 6 days in conditioned medium of mouse myeloid leukemia M1 cells with phytohemagglutinin were induced to differentiate predominantly into monocytes and macrophages (M+Ms) in a concentration-dependent manner in concentrations up to 50% (vol/vol) of the conditioned medium. The interferons alone neither inhibited HL-60 cell growth nor induced differentiation, but both enhanced growth inhibition and induction of differentiation into M+Ms by suboptimal concentrations the conditioned medium of M1 cells. The interferons also increased macrophage inducing power of low concentrations of 12-O-tetradecanoylphorbol-13-acetate and the granulocyte inducing effect of retinoic acid. The mechanism of IFA and IFB action is not known, but it seems to differ from that of cerebrospinal fluid. (27 Refs)

22. PURIFICATION AND PROPERTIES OF A RAT LIVER PROTEIN THAT SPECIFICALLY INHIBITS THE PROLIFERATION OF NONMALIGNANT EPITHELIAL CELLS FROM RAT LIVER.

McMahon JB, Farrelly JG, Iype PT
(c/o Iype) Chemical Carcinogenesis Program, Frederick Cancer Res. Center, Frederick, MD, 21701
Proc Natl Acad Sci USA; 79(2):456-460 1982

An inhibitor of cell proliferation was purified from rat liver by alcohol precipitation, ultrafiltration, and DEAE-cellulose chromatography. The hepatic proliferation inhibitor was shown to be pure by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate, analytical isoelectric focusing, and high-performance liquid chromatography. The hepatic proliferation inhibitor was found to have a molecular weight of 26,000 and an isoelectric point of 4.65. This protein inhibited the proliferation of nonmalignant rat liver cells in culture, and removal of the protein reversed the inhibition produced by low doses. It exerted no effect on the proliferation of malignant rat liver cells. (Author abstract) (31 Refs)

23. PARTIAL PURIFICATION OF A LOW MOLECULAR WEIGHT INHIBITOR OF EPIDERMAL DNA SYNTHESIS.

Quirk AV, Pain RH, Davison M, Shuster S
(c/o Shuster) Dept. Dermatology, The University, Newcastle upon Tyne NE1 7RU, England
Cell Tissue Kinet; 15(1):31-38 1982

An attempt has been made to purify factors present in aqueous extracts of pig epidermis which inhibit epidermal cell proliferation. A lipophilic factor of low molecular wt (less than 10,000), has been shown to inhibit DNA synthesis as measured by the uptake of tritiated thymidine in mouse ear epidermis. Purification by alcohol precipitation, ethyl acetate extraction and silicic acid column chromatography produced a 15-fold increase in the specific activity of the inhibitory action. It seems likely that aggregation or absorption of this low molecular wt factor may explain the high molecular wt of epidermal cell proliferation inhibitors previously studied, as well as the difficulty in their characterization. (Author abstract) (12 Refs)

HORMONES/ STEROIDS/ PROSTAGLANDINS AS GROWTH EFFECTORS

24. DEMONSTRATION OF RECEPTORS FOR INSULIN AND INSULIN-LIKE GROWTH FACTORS ON SWARM RAT CHONDROSARCOMA CHONDROCYTES. EVIDENCE THAT INSULIN STIMULATES PROTEOGLYCAN SYNTHESIS THROUGH THE INSULIN RECEPTOR.

Foley TP, Nissley SP, Stevens RL, King GL, Hascall VC, Humbel RE, Short PA, Rechler MM
Children's Hosp. Pittsburgh, 125 DeSoto St., Pittsburgh, PA, 15213
J Biol Chem; 257(2):663-669 1982

The insulin-like growth factor, multiplication stimulating activity (MSA), and insulin were recently shown to stimulate proteoglycan synthesis in monolayer cultures of chondrocytes derived from the Swarm rat chondrosarcoma. Insulin produced significant stimulation at a concentration of less than 1 ng/ml, suggesting that insulin was acting through the insulin receptor rather than through a somatomedin receptor. In this paper we

have shown that the insulin-like growth factors IGF-I and IGF-II also are potent stimulators of [35S]sulfate incorporation into macromolecules recovered from the medium and cell layer matrix of the chondrosarcoma chondrocytes. Proinsulin was 3% as potent as insulin in stimulating [35S]sulfate incorporation. We identified receptors for insulin and the insulin-like growth factors, MSA, IGF-I, and IGF-II. Insulin, at concentrations 1,000 times the concentration required to produce the biologic response, did not compete for binding of 125I-MSA-II-1 or of 125I-IGF-II and only partially competed for 125I-IGF-I binding. Anti-insulin receptor IgG stimulated proteoglycan synthesis and competed for 125I-insulin binding. Fab fragment prepared from anti-insulin receptor IgG completely blocked the stimulation of [35S]sulfate incorporation into macromolecules by insulin while only partially inhibiting the biologic response to insulin-like growth factors, MSA, IGF-I, and IGF-II. Similarly, the anti-insulin receptor IgG only partially inhibited the binding of 125I-IGF-I and 125I-IGF-II while completely blocking the binding of 125I-insulin. We conclude that insulin stimulates proteoglycan synthesis in the chondrosarcoma chondrocytes by acting through the insulin receptor, whereas the insulin-like growth factors probably act through their own receptors. (Author abstract) (41 Refs)

25. INSULIN RECEPTORS IN HUMAN CANCER.

Benson EA, Holdaway IM
(c/o Holdaway) Section Endocrinology, Auckland Hosp., Auckland 1, New Zealand
Br J Cancer; 44(6):917-920 1981

A variety of human cancers were tested for specific receptors for insulin. Sixty-seven tissue samples (40 tumors, 24 fat, 3 muscle) were obtained during surgery were homogenized; the pellet contained receptors for prolactin and insulin. The insulin-binding assay was performed using porcine unlabeled and insulin labeled with 125I using chloramine-T. Specific binding was measured using Scatchard analysis. All specific binding values were adjusted to a membrane-protein concentration of 3.0 mg/ml; range of values found was 0.5-5.0 mg/ml. Significant insulin binding was detected in all tumor specimens except one breast-tumor sample which had a low yield of membrane protein of 1.5 mg/ml compared to an av value of 33.1 mg/ml. 125I-insulin binding to different types of tumor was compared with insulin binding to normal placenta, fat and muscle. Degradation of 125I-insulin by membranes from normal and tumor tissue was compared in one specimen of colon carcinoma. Little difference was detected in the rate of insulin degradation. Rate of degradation of labeled insulin also appeared similar in different types of tumor (colon, stomach, and breast). Affinity of binding decreased with increasing receptor occupancy. Apparently insulin receptors in normal cells are preserved during malignant transformation. However, other studies have shown that tumors can also synthesize ectopic hormone receptors. Results suggest that insulin binding is a common property of human cancer, and that insulin may help maintain or stimulate the growth of human tumors. (13 Refs)

26. SPECIFIC DIFFERENTIATION EVENTS INDUCED BY ERYTHROPOIETIN IN CELLS INFECTED IN VITRO WITH THE ANEMIA STRAIN OF FRIEND VIRUS.

Koury MJ, Bondurant MC, Duncan DT, Krantz SB, Hankins WD
Div. Hematology, Dept. Medicine, Vanderbilt Univ. Sch. Medicine, Veteran's Admin. Medical Center, Nashville, TN, 37232
Proc Natl Acad Sci USA; 79(2):635-639 1982

Mouse bone marrow cells infected in vitro with the anemia strain of Friend leukemia virus form large clusters (bursts) of erythroblasts after 5 days in culture in methylcellulose medium. Two types of erythroblast populations can be isolated from bursts of infected cells by manipulation of the culture conditions. One type of erythroblast, which is obtained when erythropoietin (EP) is added to the culture, has proliferated and undergoes differentiation to become an erythrocyte. The second type of erythroblast, which is obtained when no EP is added to the culture, is the product of extensive proliferation, but it fails to undergo the terminal stages of erythroblast differentiation. Comparisons of these two types of erythroblasts demonstrate that specific EP effects include changes in the nucleus, cytoplasm, and membrane of the treated cells. Those events of erythroid differentiation shown to be directed by EP were extrusion of the nucleus from the erythroblast, induction of uroporphyrinogen I synthetase activity, increased iron incorporation into protoporphyrin, synthesis of alpha- and beta-globin polypeptides due largely to increased mRNA production, and synthesis and incorporation of spectrin into the cell membrane. In this system, EP promotes these effects without observable stimulation of progenitor proliferation in addition to that caused by the virus alone. Thus, the role of EP in terminal erythrocyte differentiation is not simply that of an erythroid-specific mitogen. (Author abstract) (30 Refs)

27. ESTROGEN REGULATION OF GROWTH AND SPECIFIC PROTEIN SYNTHESIS IN HUMAN BREAST CANCER CELLS IN TISSUE CULTURE.

Edwards DP, Adams DJ, McGuire WL
Dept. Medicine, Univ. Texas Health Science Center, 7703 Floyd Curl Drive, San Antonio, TX, 78284
Adv Exp Med Biol; 138:133-149 1982

New protein markers of estrogen action and dependence of progesterone receptors upon estrogen-stimulated cell growth were studied using MCF-7 human breast cancer cells in tissue culture. Results showed that estradiol stimulates DNA polymerase activity in normal target tissues. Progesterone receptor induction and estrogen-mediated cell division may involve separate control mechanisms. A specific 24,000 mol wt protein may be a marker of estrogen-stimulated growth of human breast tumor cells. (27 Refs)

28. NEW CONCEPTS IN CONTROL OF ESTROGEN-RESPONSIVE TUMOR GROWTH.

Sirbasku DA, Topp, Hilf
Dept. Biochemistry and Molecular Biology, Univ. Texas Medical Sch. Houston, Houston, TX, 77025
Banbury Rep; 8:425-443 1981

Estrogen-dependent (ED) tumor growth in vivo was examined. The possibility that endocrine, paracrine, or autocrine mechanisms are important components of the growth of ED tumors in vivo is discussed. Evidence for estrogen induction of uterine growth factor activity, partial purification of the uterine extract mitogenic activity, and the in vivo role of uterine-derived growth factor activity are discussed. Whether a single factor from the uterus is responsible for growth of MTW9/PL and GH3/C14 cells or whether two separate but related factors exist has not been resolved. (24 Refs)

29. HORMONAL REGULATION OF HUMAN BREAST CANCER CELLS IN VITRO.

Lippman M, Meites J, Welsch CW, Rosen JM, Segaloff A, Korenman SG, Nandi S, Sirbasku DA
Medical Breast Cancer Section, Medicine Branch, NCI, Bethesda, MD, 20205
Banbury Rep; 8:171-184 1981

Cultured breast cancer cell lines (most of which were derived from neoplastic effusions, often after treatment) differ from the original tumors in several important ways. Experiments with these cell lines must be designed and their results interpreted with great caution. Firm conclusions as to the absence of a given hormone response are particularly difficult to interpret. Positive results are much less ambiguous, but these can also give rise to misinterpretations. Nevertheless, the cell-culture approach provides several advantages in the investigation of hormonal interactions with human breast cancer. In particular, drug- and hormone-resistant variants of wild-type parental cell lines can be used to study the mechanisms of hormone dependence. Hormones now known to affect the growth or differentiated functions of human breast cancer cells (especially the MCF-7 cell line) include estrogens, glucocorticoids, androgens, thyroid hormones (the ZR-75-1 cell line is absolutely dependent on physiologic levels of triiodothyronine in the medium, and MCF-7 cells are also thyroid hormone dependent), and insulin. Vitamin A derivatives are required for normal epithelial differentiated and inhibit several types of chemical carcinogenesis in rats. Several human breast cancer cell lines contain separable binding activities for retinol and retinoic acid, but clinical trials of 13-cis-retinoic acid in breast cancer patients have not been encouraging. The roles of epidermal growth factor, vitamin D, progesterone, and prolactin in breast cancer growth are not yet clear. (46 Refs)

30. PROLACTIN, PITUITARY HORMONES, AND BREAST CANCER.

Shiu RP, Lippman M, Rosen JM, Sirbasku DA, Welsch CW
Dept. Physiology, Faculty Medicine, Univ. Manitoba, Winnipeg, Manitoba R3E OW3, Canada
Banbury Rep; 8:185-196 1981

The number of prolactin receptors (PR) in 11 human breast cancer cell lines ranged from 25,800 to 1,094; there were 1,700 in a normal cell line. The biological significance of PR and biological responses produced by PR are discussed. No growth-regulatory effect of prolactin has been demonstrated. In vivo, human breast cancer cells were responsive to estrogen and pituitary hormones. (25 Refs)

31. ROLE OF HORMONES IN MAMMOGENESIS AND CARCINOGENESIS.

Nandi S, Yang J, Richards J, Guzman R, Shellabarger CJ, Rosen JM, Sirbasku DA, Silteri PK, Korenman SG
Dept. Zoology and Cancer Res. Lab., Univ. California, Berkeley, CA, 94720
Banbury Rep; 8:445-456 1981

Development of a collagen system for growing mammary cells in vitro is described. Results for general aspects of growth of mammary cells inside collagen gel matrix (CGM), growth of normal mouse mammary epithelial cells (MEC) inside CGM (effects of hormones and growth factors), and for growth of normal human MEC inside CGM (effects of hormones and tissue extracts) are presented. Explanation for the observation that mammogenic hormones are growth-promoting in vivo appears to be in the fact that hormone-induced growth of a tissue or organ in vivo is a complicated phenomenon encompassing a number of biological events at the tissue level, including cell division; hormones might be the ultimate regulators of tissue growth, although they are not directly mitogenic. (27 Refs)

32. BILATERAL ACOUSTIC NEUROMAS: CLINICAL ASPECTS, PATHOGENESIS, AND TREATMENT.

Martuza RL, Ojemann RG, Malis LI
Neurosurgical Service, Massachusetts General Hosp., Boston, MA, 02114
Neurosurgery; 10(1):1-12 1982

The cases of 15 patients (9 female, 14-74 yr old; 6 male, 12-32 yr old) with bilateral acoustic neuromas are described. The technical surgical problems, results of treatment, and complications of this illness are discussed. Eleven of the patients had no family history of neurofibromatosis or CNS tumor, and a spontaneous mutation is considered to be responsible for the initiation of disorders in thes e patients. Most of the patients were 21 yr old or younger when their symptoms began; they had a more fulminant course and a higher incidence of multiple CNS tumors than those who developed symptoms later. Evidence that 17-beta-estradiol may be an important part of the tumor growth control mechanism is presented, and it may explain why Schwann cell tumors preferentially occur in the vestibular nerve, trigeminal nerve, and spinal sensory nerves. Genetic aspects and potential treatment alternatives of bilateral acoustic neuromas are discussed. (51 Refs)

33. THE INFLUENCE OF ANDROGENS, ANTI-ANDROGENS, AND CASTRATION ON CELL PROLIFERATION IN THE JEJUNAL AND COLONIC CRYPT EPITHELIA, AND IN DIMETHYLHYDRAZINE-INDUCED ADENOCARCINOMA OF RAT COLON.

Tutton PJ, Barkla DH
Dept. Anatomy, Monash Univ., Clayton, 3168, Australia
Virchows Arch [Cell Pathol]; 38(3):351-355 1982

Androgenic hormones have previously been shown to promote cell proliferation in the small intestine of rat and androgen receptors have been demonstrated in carcinomata of the large intestine of rat. In this study the influence of testosterone and of castration on epithelial cell proliferation in the small intestine, the large intestine and in dimethylhydrazine-induced colonic tumors is compared. Cell proliferation in the small intestine and in colonic tumors was accelerated by testosterone treatment, and cell proliferation in colonic tumors, but not in the small intestine, was retarded following castration. Cell proliferation in colonic tumors was also inhibited by the anti-androgenic drug, Flutamide. Testosterone and castration each failed to influence cell proliferation in the colonic crypt epithelium of both normal and carcinogen-treated animals. (Author abstract) (22 Refs)

34. MODULATION OF LEUKAEMIA BLAST COLONY GROWTH BY STEROID HORMONES.

Taetle R, Guittard JP
Univ. Hosp. H720, 225 Dickinson St., San Diego, CA, 92103
Br J Haematol; 50(2):247-255 1982

The effect of steroid hormones on in vitro colony growth of human myelogenous leukemia cells was examined. Beta-estradiol,testosterone and 5-beta-dihydrotestosterone had little effect on HL60 promyelocytic leukemia cells or blast colony forming cells (CFC) from eight patients with acute non-lymphocytic leukemia (ANLL). These compounds inhibited blast colony growth at very high concentrations (10(-6)-10(-4) M).

In contrast, hydrocortisone (HC) had highly variable effects on blast colony growth. HL60 cells were resistant to HC at concentrations up to 10(-4) M but blast CFC showed a wide range of response. Some patients were resistant to HC at all concentrations tested, while others were inhibited by concentrations as low as 10(-8) M. The inhibitory effect of HC was also observed on 3H-TdR incorporation by stimulated peripheral blood blasts. Inhibition by HC was not blocked by progesterone, suggesting this effect was not mediated by specific hormone receptors. These data indicate differences in the response of blast cells in ANLL and normal progenitors to steroid hormones. Cells from patients with ANLL display variable inhibition by HC which is probably not mediated by specific receptors. (Author abstract) (30 Refs)

35. EXPRESSION OF IA-ANTIGENS ON NORMAL AND CHRONIC MYELOID LEUKEMIC HUMAN GRANULOCYTE-MACROPHAGE COLONY-FORMING CELLS (CFU-GM) IS ASSOCIATED WITH THE REGULATION OF CELL PROLIFERATION BY PROSTAGLANDIN E.

Pelus LM, Saketan S, Silver RT, Moore MA

Dept. Developmental Hematopoiesis, Sloan-Kettering Inst. Cancer Res., 1250 First Ave., New York, NY, 10021

Blood; 59(2):284-292 1982

The presence of Ia-antigens and their relationship to the inhibitory effect of prostaglandin E on the proliferation of human CFU-GM was studied in normals and patients with chronic myeloid leukemia. Consistent reduction of normal colony formation to approx 50% of baseline levels was observed using a monoclonal anti-human Ia antibody in a complement-dependent cytotoxicity assay titrated over serial dilutions. Elimination of the Ia-antigen-bearing CFU-GM population was associated with virtually a complete loss of responsiveness to the inhibitory effect of prostaglandin E. Maintenance of bone marrow cells in short-term suspension culture at 37 C prior to agar culture resulted in the loss of detectable Ia-antigen on the CFU-GM and, similarly, loss of response to prostaglandin. In contrast, most patients with chronic myeloid leukemia showed greatly reduced levels of Ia-antigens on their CFU-GM in fresh marrow together with lack of prostaglandin sensitivity, suggesting a correlation with the abnormal growth regulation observed in these patients. In two chronic myeloid leukemia patients, levels of Ia-antigen higher than that observed in the majority of patients could be detected and correlated with a residual response to prostaglandin E. These results suggest a relationship in normals between the expression of Ia-antigens on CFU-GM and the physiologic response to regulation by prostaglandin E, and a possible mechanism for the aberrant regulatory response in patients with chronic myeloid leukemia. (Author abstract) (41 Refs)

CYCLIC NUCLEOTIDE/ POLYAMINES/ NUCLEOTIDES AS GROWTH EFFECTORS

36. THE PRIMARY TISSUE CULTURE OF HUMAN ADRENOCORTICAL CONN'S ADENOMATA. I. THE SYNERGISTIC STIMULATION OF ADENOMATOUS CELL GROWTH BY PURINE CYCLIC NUCLEOTIDES AND BY ACTH1-24 AND ANGIOTENSIN II.

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Pathol Res Pract; 173(1/2):66-81 1981

Primary cultures of dissociated parenchymal cells from Conn's adenomata causing primary hyperaldosteronism were successfully set up by a method previously used with normal adult human and rat adrenocortical tissue. In such cultures the adenomatous cells largely prevailed (making up 87% of the whole cell population), could survive for lengthy terms (at least up to 30 days), and were endowed with a spontaneous, discrete capability to proliferate. The de novo RNA- and DNA-synthetic and mitotic activities of Conn's cells were markedly stimulated in cultures exposed between day 16 and 21 to daily doses of exogenous cyclic AMP, either alone or in equimolar association with cyclic GMP. A significantly weaker, though still prominent enhancement of adenomatous cell growth was elicited also by daily administrations of an equimolar mixture of ACTH1-24 and angiotensin II. In contrast, little stimulation or inhibition of growth or no effect at all could be observed when cyclic GMP, ACTH1-24, and angiotensin II were respectively administered, each by itself. The present results support the working hypothesis that Conn's adenomatous cell growth is orderly controlled by several hormonal factors, including angiotensin and ACTH, the actions of which are mediated by both cyclic AMP and GMP. (Author abstract) (39 Refs)

37. EFFECTS OF CYCLIC AMP ON GROWTH AND DIFFERENTIATION OF RAT RETINOBLASTOMA-LIKE TUMOR CELLS IN VITRO.

Nishida T, Mukai N, Solish SP, Pomeroy M

Dept. Ophthalmology, Osaka Univ. Medical Sch., 1-1-50 Fukushima, Fukushima-Ku, Osaka 553, Japan

Invest Ophthalmol Vis Sci; 22(2):145-156 1982

Effects of cyclic AMP analogs and phosphodiesterase of RAO 188 cells were investigated. The cell line was derived from a retinoblastoma-like rat tumor. 14C-Thymidine and 3H-uridine incorporation by RAO cells was inhibited by 1 milliM dibutyryl cyclic AMP, and inhibition reached a maximum at 5 milliM. Precursor incorporation was also inhibited by 8-bromocyclic AMP, theophylline, and aminophylline, but not by dibutyryl cyclic guanosine monophosphate. Further observations indicated that dibutyryl cyclic AMP inhibited both DNA and RNA synthesis and that inhibition was irreversible after 10 days of incubation. Neurite-like processes developed shortly after cyclic AMP analog treatment and formed a network after 24 hr; ultrastructure was well organized and characterized by microvillous projections and intercellular junctional complexes. Results show that treatment which increases intracellular cyclic AMP concentration halts growth of RAO rat retinoblastoma-like tumor cells in culture and facilitates their morphologic and biochemical differentiation. (21 Refs)

COFACTORS/ NUTRITIONAL GROWTH FACTORS

38. EFFECT OF RETINOIC ACID TREATMENT OF F9 EMBRYONAL CARCINOMA CELLS ON THE ACTIVITY AND DISTRIBUTION OF CYCLIC AMP-DEPENDENT PROTEIN KINASE.

Piet A, Evain D, Anderson WB
Unite Institut National de la Sante et de la Recherche Medicale 188, 75014 Paris, France
J Biol Chem; 257(2):889-893 1982

Treatment of F9 teratocarcinoma cells with all trans-retinoic acid previously has been shown to induce differentiation to parietal endoderm and to enhance the responsiveness of these cells to the addition of cyclic AMP. Evidence is presented that retinoic acid treatment of F9 cells causes an increase in both cytosolic- and plasma membrane-associated cyclic AMP-dependent protein kinase activities. Retinoic acid elicits a progressive increase in cytosolic kinase activity through 72 hr. Cyclic AMP-dependent kinase activity of the plasma membrane fraction is enhanced within 3 hr of retinoid treatment of F9 cells and reaches a maximum at 15 hr after the addition of retinoic acid. The amount of regulatory subunits of cyclic AMP-dependent protein kinases I (RI) and II (RII) present in the subcellular fractions was quantitated by photoaffinity labeling with 8-azido-cyclic [32P]AMP. In the cytosol, the amount of RI and RII both increase with the period of retinoid treatment through 24 hr, and remain elevated through 5 days; the ratio of RI/RII (approx 2.5) found in the cytosol remains constant following retinoic acid treatment. RI is much more prevalent than RII (RI/RII = 7.5) in the plasma membrane prepared from untreated F9 cells. Retinoic acid treatment elicits a preferential increase in the amount of the RII regulatory subunit associated with the membrane fraction. After 15 hr of retinoid treatment, a 1.6-fold increase in RI is observed, while membrane-associated RII is increased 4.5-fold. After 24 hr of retinoic acid treatment, the level of RI returns toward the level noted with untreated cells, while the amount of RII remains significantly elevated through 5 days of retinoid treatment. These findings suggest that the increase in cyclic AMP-dependent protein kinase activity and the specific accumulation of RII associated with the plasma membrane fraction may be early events of retinoic acid action to mediate eventual cellular differentiation. (Author abstract) (38 Refs)

39. REVERSIBLE AND IRREVERSIBLE STAGES IN THE TRANSITION OF CELL SURFACE MARKER DURING THE DIFFERENTIATION OF PLURIPOTENT TERATOCARCINOMA CELL INDUCED WITH RETINOIC ACID.

Ogiso Y, Kume A, Nishimune Y, Matsushiro A
Dept. Microbial Genetics, Res. Inst. Microbial Diseases, Osaka Univ., Yamada-oka 3-1, Suita Osaka 565, Japan
Exp Cell Res; 137(2):365-372 1982

Treatment of a pluripotent teratocarcinoma cell line with retinoic acid (RA) results in the disappearance of peanut agglutinin (PNA) receptors, accompanied with the decrease in F9 antigens and the enhanced secretion of plasminogen activator. However, this type of differentiation was inhibited by feeder cells. Furthermore, the transition of PNA receptor was reversible on the cells treated with RA for 2 days and became irreversible by an additional 2-day treatment with RA. Thus, two stages of teratocarcinoma cell differentiation - reversible and irreversible - were demonstrated. (Author abstract) (22 Refs)

40. RELATIONSHIP OF IRON METABOLISM TO TUMOR CELL TOXICITY OF STABLE GALLIUM SALTS.

Rasey JS, Nelson NJ, Larson SM
Dept. Radiation Oncology, Univ. Washington, Seattle, WA
Int J Nucl Med Biol; 8(4):303-313 1981

The antitumor properties of citrated gallium nitrate (NSC 15200) have been tested using the EMT-6/UW mouse sarcoma growing in vitro or in vivo. This tumor system has been used in both growth forms in our studies which led to the transferrin receptor hypothesis of the mechanism of uptake of tracer levels of gallium-67 citrate. Gallium nitrate is both cytostatic and lethal in vitro with some growth inhibition occurring after chronic exposure to low doses (10 ug per ml) which kill essentially no cells. Cell kill and growth inhibition were both observed if cells were exposed for longer times at higher doses. The effects of gallium nitrate were enhanced by the addition of human transferrin to the culture medium. The enhanced toxicity was consistent with, and proportional to, increased gallium uptake in the presence of transferrin. The addition of ferric citrate greatly reduced the toxic effect of the gallium salt. The enhancing effect of transferrin and protective action of ferric citrate are consistent with our studies of the effect of these two agents on concentration of tracer levels of the tumor-imaging radiopharmaceutical, carrier-free gallium-67 citrate. Tumors in vivo were largely resistant to 10 daily doses of 90 mg kg-1 of gallium nitrate. Ga may mimic Fe in some aspects of cell metabolism. Competition between the two metals occurs at the initial uptake step (binding to transferrin) and possibly to a lesser extent at other points in cellular incorporation and deposition. Iron has been implicated as a regulatory factor in cell growth and may be essential for cell proliferation. The toxicity of stable gallium is interpreted in the context of similarities of metabolism of the two metals. (Author abstract) (20 Refs)

41. EFFECT OF L-METHIONINE DEPRIVATION ON THE PROLIFERATIVE ACTIVITY OF NORMAL AND LEUKAEMIC MARROW CELLS IN VITRO.

Eridani S, Sawyer B, Villa S, Tisdale M
St. Thomas's Hosp. and Medical Sch., London SE1 7EH, England
J Clin Hematol Oncol; 12(1):9-15 1982

Following suggestions that some tumor cells may have a high requirement for methionine, a comparative study has been carried out between normal and leukemic bone marrow (obtained from patients with ALL, AML and CML) with regard to the capacity to incorporate (methyl-3H) thymidine into acid-insoluble material, after incubation in media lacking L-methionine and supplemented with L-homocysteine. A lower incorporation was shown by leukemic cells, as compared with normal ones, with a significant differ ence (p less than 0.05). This difference is not due to inability by leukemic cells to synthesize L-methionine from homocysteine, because an increased incorporation can be seen in the presence of homocysteine when methionine concentration in culture becomes limiting. Further experiments suggest that such high methionine requirement might be due to a decreased transport capacity by leukemic cells: all marrows show the same linear uptake for the whole range of concentrations tested, but the maximum uptake velocity is lower in leukemic cells. Induction by different means of a restricted supply of methionine to leukemic cells might play a role in the control of myeloproliferative disorders. (Author abstract) (11 Refs)

42. A CHEMICALLY DEFINED MEDIUM FOR THE PROPAGATION OF RAT PROSTATE ADENOCARCINOMA CELLS.

Chan SY
Lobund Lab., Univ. Notre Dame, South Bend, IN, 46556
Prostate; 2(3):291-298 1981

A serum-free growth medium, MB-DF, consisting of a modified Waymouth's MB-752/1 medium supplemented with fetuin (150 ug/ml), insulin (5 ug/ml), transferrin (25 ug/ml), dexamethasone (100 nanog/ml), 5alpha-dihydrotestosterone (100 nanog/ml), NaHCO3 (1.1 mg/ml), penicillin (100 units/ml), and streptomycin (100 ug/ml) was developed and successfully tested with rat prostate adenocarcinoma cell line PA-III derived from aging Lobund Wistar rats. The 95th passage of PA-III was adapted to culture by gradually decreasing the amount of fetal bovine serume (FBS) in the medium from 10% to zero. The adapted cell line PA-IIIf, stored up to 12 mo at-70 C, still exhibited excellent recovery of viable propagating cells. PA-IIIf cells retained the same tumorigenicity as the parent line, and metastasized via the same ipsilateral lymphatic pathway from the footpad to the lungs, in which they produced distinct metastatic foci. (21 Refs)

EXOGENOUS AGENTS AFFECTING DIFFERENTIATION

43. INDUCTION OF DIFFERENTIATION OF HUMAN PROMYELOCYTIC LEUKEMIA (HL-60) CELLS BY TELEOCIDIN AND PHORBOL-12-MYRISTATE-13-ACETATE.

Huberman E, Braslawsky GR, Callaham M, Fugiki H
Biology Div., Oak Ridge Natl. Lab., Oak Ridge, TN, 37830
Carcinogenesis; 3(1):111-114 1982

Human promyelocytic leukemia cells (HL-60) were induced to differentiate into macrophage-like cells in a dose-dependent manner by the tumor promoters phorbol-12-myristate-13-acetate and teleocidin, a non-phorbol ester promoter. An HL-60 cell variant, designated as R-59, which is resistant to differentiation induction by phorbol-12-myristate-13-acetate was also resistant to differentiation induction by teleocidin. Differentiation was determined by increases in the percent of morphologically mature cells and in lysozyme and nonspecific esterase activities. Both compounds inhibited the growth of HL-60 cells by blocking them from entering the synthesis phase of the cell cycle with an accumulation of cells aft er 48 hr in G1 phase. No such effects were observed in the R-59 cells. They were, however, as susceptible as the parent HL-60 cells, to inducers which are not considered to be tumor promoters such as dimethylsulfoxide and retinoic acid. However, these inducers cause the HL-60 and R-59 cells to differentiate into granulocyte-like cells. These results indicate that teleocidin produces in both the HL-60 and R-59 cells effects which are similar to those caused by phorbol-12-myristate-13-acetate. The possibility that agents producing such effects in these two cell types may represent potential tumor promoters is discussed. (Author abstract) (28 Refs)

44. LIPID COMPOSITION OF FRIEND LEUKEMIA CELLS FOLLOWING INDUCTION OF ERYTHROID DIFFERENTIATION BY DIMETHYL SULFOXIDE.

Rittmann LS, Jelsema CL, Schwartz EL, Tsiftsoglou AS, Sartorelli AC
(c/o Sartorelli) Dept. Pharmacology, Yale Univ. Sch. Medicine, Comprehensive Cancer Center, New Haven, CT, 06510
J Cell Physiol; 110(1):50-55 1982

The rates of incorporation of (14C)glycerol (14CG) and 3H-methyl choline (3HMC) into Friend erythroleukemia cells, both untreated and exposed to 1.5% dimethyl sulfoxide (DMSO), were determined. Both 14CG and 3HMC were incorporated linearly into lipids for up to 240 min; DMSO did not alter incorporation over the time period studied. Except for decreases in the proportion of sphingomyelin and diphosphatidylglycerol and a slight increase in phosphatidyl ethanolamine, phospholipids of cells differentiated by exposure to DMSO did not differ from control cells. The fatty-acid chain length did not appear to change substantially in differentiated cells, but a 15.6% decrease in saturation in the neutral lipid fraction and a 3% saturation decrease in the phospholipid fraction in DMSO-treated cells were noted. DMSO-induced cultures contained significantly less free and esterified cholesterol than did the individual control cells. The initial event involved in DMSO-induced differentiation of Friend leukemia cells apparently does not involve a change in phospholipid synthesis; that observed changes in membrane fluidity appear not to be due to changes in overall fatty-acid chain length in total neutral lipids and phospholipids. The effect of DMSO on cholesterol content indicates that maturation of the murine erythroleukemia cells is related to alterations in the cholesterol:phospholipid ratio. (48 Refs)

45. SYNTHESIS OF SIALOGLYCOCONJUGATES DURING DIMETHYLSULFOXIDE-INDUCED ERYTHRODIFFERENTIATION OF FRIEND LEUKEMIA CELLS.

Brown AE, Schwartz EL, Dreyer RN, Sartorelli AC
Brown,, (c/o Sartorelli) Comprehensive Cancer Center, Yale Univ. Sch. Medicine, New Haven, CT, 06510
Biochim Biophys Acta; 714(2):217-225 1982

We have previously shown that erythroid differentiation of Friend murine leukemia cells by dimethylsulfoxide results in a decrease in sialic acid content and net negative surface charge. The mechanism responsible for the decrease in sialic acid content was examined by measuring the synthesis of sialic acid from N-acetylmannosamine and its catabolic removal from sialoconjugates during the maturation process. A decrease in the incorporation of N-[3H]acetylmannosamine into sialoglycoconjugates occurred as early as 12 hr after exposure to dimethylsulfoxide. Radioactivity incorporated into sialoglycoconjugates was relatively stable in untreated and dimethylsulfoxide-treated cells, implying that catabolic removal of sialic acid residues was not a factor in the decreased surface sialic acid content of differentiated erythroleukemia cells. In addition, no difference existed between control and treated cells in sialyltransferase activity. Significant decreases occurred, however, in the incorporation of radioactivity from N-[3H]acetylmannosamine into N-acetylneuraminic acid, CMP-N-acetylneuraminic acid and a material tentatively identified as N-acetylmannosamine-6-phosphate, 48 hr after the addition of dimethylsulfoxide. The decrease in sialic acid biosynthesis in differentiated erythroleukemia cells was reflected by an 83% decrease in the amount of radioactivity-labeled sialic acid released by neuraminidase treatment of cells exposed to dimeth-

ylsulfoxide. These findings are consistent with a cellular aging phenomenon triggered by the polar solvent-induced differentiation of the leukemic cells into more mature forms. (Author abstract) (30 Refs)

46. INDUCER-MEDIATED COMMITMENT OF MURINE ERYTHROLEUKEMIA CELLS TO DIFFERENTIATION: A MULTISTEP PROCESS.

Chen ZX, Banks J, Rifkind RA, Marks PA
Soochow Medical Coll., Soochow, People's Republic of China
Proc Natl Acad Sci USA; 79(2):471-475 1982

There are a number of agents which, when added to cultures of murine erythroleukemia cells (MELC), markedly increase the probability of commitment to express the characteristics of terminal erythroid differentiation, including loss of proliferative capacity and increased accumulation of globin mRNA and hemoglobin. Some characteristics of inducer-mediated commitment of MELC to terminal erythroid differentiation were examined by determining the effects of dexamethasone (an inhibitor of inducer-mediated MELC differentiation) and of hemin (an inducer of globin mRNA accumulation). Previously, it was shown that exposure of MELC to hexamethylene-bisacetamide (HMBA) leads to commitment, detectable within 12 hr. MELC cultured with both HMBA and dexamethasone do not express commitment. MELC transferred from culture with HMBA and dexamethasone to cloning medium without these agents express commitment to terminal erythroid differentiation, indicating that MELC retain a 'memory' for some early HMBA-mediated changes leading to commitmen t which occur even in the presence of the inhibitory steroid. The kinetics of commitment in experiments in which exposure to HMBA is interrupted, or dexamethasone is added to the culture with HMBA, suggest that there is a rate-limiting step early in the commitment process. The memory for this step persists for more than one cell cycle. Addition of hemin to cultures with HMBA and dexamethasone initiates accumulation of globin mRNA but does not reverse the steroid-mediated inhibition of terminal cell division (that is, the cells retain their proliferative capacity). Inducer-mediated MELC commitment is associated with accumulation of the chromatin protein IP25; dexamethasone does not inhibit this accumulation. Accumulation of IP25 may be inducer-related, but it is not sufficient to cause expression of terminal erythroid differentiation. (Author abstract) (49 Refs)

47. EVIDENCE THAT A NA+/CA++ ANTIPORT SYSTEM REGULATES MURINE ERYTHROLEUKEMIA CELL DIFFERENTIATION.

Smith RL, Macara IG, Levenson R, Housman D, Cantley L
Dept. Biochemistry and Molecular Biology, Harvard Univ., Cambridge, MA, 02138
J Biol Chem; 257(2):773-780 1982

The Na+ and Ca++ transport properties of cultured murine erythroleukemia (MEL) cells have been investigated. We have previously shown that amiloride prevents dimethyl sulfoxide-induced MEL cell differentiation via inhibition of an essential Ca++ influx. Here we show that external Na+ inhibits Ca++ influx and stimulates Ca++ efflux from uninduced MEL cells. Increasing the internal Na+ concentration by a brief incubation of cells with ouabain stimulates the rate of 45Ca++ influx. Amiloride (40 uM) completely blocks the external Na+-stimulated 45Ca++ efflux and external Na+-inhibitable 45Ca++ influx. The same concentration of amiloride had no significant effect on net Na+ uptake. These results suggest that a significant fraction of Ca++ flux across the MEL cell plasma membrane occurs via a Na+/Ca++ antiport system and that amiloride prevents differentiation by blocking Ca++ influx through this system. The importance of a Na+/Ca++ antiport system for MEL cell differentiation is supported by the following observation: increasing the cellular Na+ level by a brief treatment with ouabain plus monensin accelerates MEL cell commitment as effectively as adding the Ca++ ionophore A23187. We suggest that dimethyl sulfoxide induces MEL cell differentiation by inhibiting the Na+ pump and consequently allowing Ca++ influx through the Na+/Ca++ antiport. (Author abstract) (37 Refs)

48. HISTIOCYTIC DIFFERENTIATION OF MOUSE MALIGNANT FIBROUS HISTIOCYTOMA-LIKE TUMOR CELLS BY CULTURING WITH SODIUM BUTYRATE.

Tanaka H, Mori Y, Akedo H
Dept. Cell Biology, Center for Adult Diseases, Osaka 1-3-3, Nakamichi, Higashinari, Osaka 537, Japan
Cell Biol Int Rep; 6(1):85-90 1982

The histiocytic origin of the mouse malignant fibrous histiocytoma-like tumor was strongly suggested from butyrate-induction studies of cultured cells. Fc and C3 receptors and lysosomal enzyme activities were significantly induced in the two MFH cell lines studied 4 days after the addition of butyrate. The application of these results to the histogenesis of human malignant histiocytoma is briefly discussed. (10 Refs)

49. MACROPHAGE ACTIVATION BY TUFTSIN AND MURAMYL-DIPEPTIDE.

Bruley-Rosset M, Florentin I, Mathe G
Institut de Cancerologie et d'Immunogenetique, Hopital Paul-Brousse, 94800 Villejuif, France
Mol Cell Biochem; 41:113-118 1981

Peritoneal macrophages from tuftsin or MDP-treated mice were tested for their cytostatic activity for tumor cell proliferation. Both substances are able to activate macrophages either after intravenous injection or after incubation in vitro with normal macrophages. But a stimulation as well as an inhibition of tumor cell growth can result from macrophage activation depending on the timing and dose injected. Restoration of the impaired cytostatic capacity of macrophages of mice observed with aging, is obtained by repeated administration of tuftsin. Normal and BCG-stimulated macrophages were examined for their regulatory activity on the proliferation of P815 tumor cells. Low density of macrophages per well determines a stimulation of target cell growth whether the macrophages are normal or activated. When the number of macrophages is increased, under conditions in which normal macrophages are not inhibitory. BCG-stimulated macrophages exert already a strong cytostatic activity. At high macrophage content it appears that normal macrophages can also display an inhibitory activity. Macrophage-tumor cell interactions are highly dependent on the concentrations and the state of activation of macrophages. (Author abstract) (20 Refs)

OTHER RELATED STUDIES

50. MEMBRANE TRANSPORT PROPERTIES DIFFER FOLLOWING RETURN OF SERUM-DEPRIVED VERSUS CA++-DEPRIVED HUMAN FIBROBLASTS TO A PROLIFERATIVE STATE.

Tupper JT, Ryals WT, Bodine PV
Dept. Biology, Syracuse Univ., Syracuse, NY, 13210
J Cell Physiol; 110(1):29-34 1982

The unidirectional influxes of Rb+, an analog for K+ transport, and of 3-O-methylglucose, cycloleucine, and alpha-aminoisobutyric acid were monitored following stimulation of quiescent WI38 human lung fibroblast cells by Ca++ or fetal bovine serum. Quiescence in the cells had been induced earlier by deprivation of one of the same two stimulants. The absence of Ca++ at the time of serum stimulation inhibited the increase in transport usually observed upon serum stimulation, but Ca++ stimulation did not stimulate transport in Ca++-deprived cells yet still caused cells to progress to S phase. Ca++ and serum inhibition o f cell division apparently have different mechanisms. (34 Refs)

51. PEPTIDE AND PROTEIN COMPLEXES OF TRANSITION METALS AS MODULATORS OF CELLULAR REPLICATION.

Fernandez-Pol JA
Nuclear Medicine Lab., Veterans Admin. Medical Center, St. Louis, MO, 63125
Int J Nucl Med Biol; 8(4):231-235 1981

Chelation of transition metal ions with transferrin, siderophore-like growth factor (SGF) and with other potential growth-modulating proteins and peptides appears to provide a mechanism for the regulation of cell duplication by regulation of metal ion availability. Growth-modulating peptides, like SGF, synthesized and secreted by some transformed cells, may be made to take advantage of local conditions, decrease the dependence on serum factors (eg, transferrin) and to compete with normal cells for specific trace metal ions. Detailed knowledge of transition metal ion metabolism in normal and transformed cells may lead to the development of specific oncophilic radiopharmaceuticals. (Author abstract) (19 Refs)

52. THREE-DIMENSIONAL GROWTH AND MORPHOGENESIS OF MOUSE SUBMANDIBULAR EPITHELIAL CELLS IN SERUM-FREE PRIMARY CULTURE.

Yang J, Larson L, Nandi S
Cancer Res. Lab., Dept. Zoology, Univ. California, Berkeley, CA, 94720
Exp Cell Res; 137(2):481-485 1982

Mouse submandibular epithelial cells can be grown in primary culture using the collagen gel matrix and a chemically defined medium consisting of insulin, transferrin, cholera toxin, and BSA (or FGF). Sustained cell growth leading to a 5-10-fold increase in cell number was observed in less than 2 wk. In the presence of these additives, clumps of cells proliferate by extending 'star-like' projections into the matrix, resulting in three-dimensional outgrowths. The morphology of these outgrowths can be modulated to form a 'cyst-like' appearance by deleting BSA and adding cortisol to the basal medium containing insulin, transferrin, cholera toxin and FGF. In brief, a serum-free medium for sustained growth has been devised and a simple manipulation of supplements can modulate the three-dimensional colony morphology in the collagen gel matrix. Finally, the resulting outgrowths can produce epidermal growth factor (EGF) in response to dihydrotestosterone. (Author abstract) (14 Refs)

53. STEM CELL REGULATION IN ERYTHROPOIESIS.

Harrison PR
Beatson Inst. for Cancer Res., Switchback Road, Bearsden, Glasgow G61 1BD, Scotland
Nature; 295(5849):454-455 1982

Stem cell regulation in erythropoiesis is reviewed. According to the studies the earliest precursor cells and the various intermediate committed cell precursors require growth factors to sustain their proliferation and differentiation. The effects of the tissue environment on differentiation of hematopoietic stem cells could be explained by cellular contacts involved as well as the circulating growth fac tors, or the lability of essential factors acting over very short distances. (8 Refs)

54. EXPRESSION OF RED CELL SPECIFIC DETERMINANTS DURING DIFFERENTIATION IN THE K562 ERYTHROLEUKAEMIA CELL LINE.

Horton MA, Cedar SH, Edwards PA
Dept. Haematology, St. Bartholomew's Hosp., London, EC1A 7BE, England
Scand J Haematol; 27(4):231-240 1981

A human hemopoietic cell line (K562) which exhibits various erythroid characteristics, has been utilized as a model system for studying erythropoietic differentiation. We have analyzed the alterations in expression of cell membrane determinants which accompany the induction of Hb synthesis. We confirm that the K562 cell line exhibits a number of erythroid features: it expresses immunologically detectable membrane proteins, glycophorin A and spectrin, and can be induced, by addition of hemin or n-butyrate, to synthesize Hb. N and i-like blood group activities are demonstrable in uninduced K562, but band 3, ABH and other major alloantigens characteristic of mature erythrocytes are lacking. The acquisition of cell surface antigens typifying erythroid or other hemopoietic lineages has not been demonstrated following induction, although certain properties not associated with morphologically recognizable red cell precursors are lost. The relevance of these findings to the fetal nature and possible multipotentiality of the K562 cell line are discussed. (Author abstract) (25 Refs)

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CELL AND SERUM DERIVED GROWTH EFFECTORS

1. RESOLUTION OF HIGH AND LOW AFFINITY EPIDERMAL GROWTH FACTOR RECEPTORS. INHIBITION OF HIGH AFFINITY COMPONENT BY LOW TEMPERATURE, CYCLOHEXIMIDE, AND PHORBOL ESTERS.

King AC, Cuatrecasas P
Dept. Molecular Biology, Wellcome Res. Lab., Research Triangle Park, NC, 27709
J Biol Chem; 257(6):3053-3060 1982

Receptors for the mitogen epidermal growth factor (EGF) have been implicated as mediators of cellular transformation and tumor promotion. 12-O-Tetradecanoylphorbol-13-myristate

(PMA) causes immediate but transient reductions in the ability of human KB cells to internalize EGF by decreasing the affinity of cell surface EGF receptors 7-to 12-fold. After 90 min of PMA exposure, complete recov ery of the high affinity apparatus occurs. Evidence is presented that the decreased binding capacity to 125I-EGF is caused by delaying the appearance at the plasma membrane of cryptic receptor sites of high affinity. Exposure of these high affinity binding sites is temperature dependent and is blocked by pretreatment of cells with inhibitors of de novo protein synthesis. After short incubation periods (0 to 60 min) with 125I-EGF, up to 90% of the internalized 125I-EGF can be rapidly dissociated from cells by the addition of PMA. This treatment kinetically reveals a trypsin-insensitive, nondissociating component of cell-associated 125I-EGF. With PMA, both the rate (maximal at 4 hr with PMA as compared to 24 hr) and extent (8-fold greater with PMA) of transfer of 125I-EGF into this trypsin-stable compartment is enhanced and accounts for the severalfold stimulation in 125I-EGF accumulation. This evidence suggests that the internalization of 125I-EGF can be resolved into two components, one into which EGF immediately partitions and is freely exchangeable with the extracellular medium and that is disrupted by PMA. A second and previously unknown compartment retains 125I-EGF in the presence of PMA after a short delay to a considerably greater extent than that of untreated cells. Thus, without PMA, EGF is internalized into this nondissociating intracellular compartment, but the transfer is only 5-10% as efficient. These results suggest that the accumulation of stable intracellular mitogen-receptor complexes may be directed by a population of very high affinity receptors which may be an element involved in the control of cellular growth and tumor promotion. (Author abstract) (46 Refs)

2. EPIDERMAL GROWTH FACTOR AND INSULIN CAUSE NORMAL CHICKEN HEART MESENCHYMAL CELLS TO PROLIFERATE LIKE THEIR ROUS SARCOMA VIRUS-INFECTED COUNTERPARTS.

Balk SD, Shiu RP, LaFleur MM, Young LL
Lab. Pathology, New England Deaconess Hosp., 185 Pilgrim Road, Boston, MA, 02215
Proc Natl Acad Sci USA; 79(4,part1):1154-1157 1982

Normal chicken heart mesenchymal cells at low culture density are proliferatively quiescent in a physiological culture medium containing heparinized, heat-inactivated, chicken plasma at 10%. Rous sarcoma virus-infected chicken heart mesenchymal cells, on the other hand, proliferate maximally in this same medium, undergoing a 60-fold increase in cell number during 4 days of exponential growth. When normal heart mesenchymal cells are cultured for 4 days in the presence of epidermal growth factor at 1 ug/ml they undergo a 16-fold increase in number, with graded responses to lower concentrations of the factor. In the presence of insulin at 10 ug/ml, normal heart mesenchymal cells multiply by a factor of 3 over a 4-day period. The addition of epidermal growth factor (1 ug/ml) and insulin (10 ug/ml) to cultures of normal chicken heart mesenchymal cells causes these cells to proliferate at a rate comparable to that of their RSV-infected counterparts. (Author abstract) (22 Refs)

3. MITOGENIC EFFECT OF EPIDERMAL GROWTH FACTOR ON SHEEP THYROID CELLS IN CULTURE.

Westermark K, Westermark B
Dept. Internal Medicine, Univ. Uppsala, S-751 22 Uppsala, Sweden
Exp Cell Res; 138(1):47-55 1982

Sheep thyroid follicles enzymatically released from fragmented tissue were used for primary and clonal monolayer culture. Bovine thyrotropin in combination with the phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine (MIX), induced morphological differentiation with the formation of follicular lumina. An autoradiographical analysis of the incorporation of [3H]thymidine in primary cultures showed that epidermal growth factor (EGF) caused an increase (about 50%) in the labeling index. Further, it was shown that EGF, when combined with a low serum concentration in Eagle's minimum essential medium (EMEM), allowed clonal growth of epithelial cells, whereas high serum concentration governed growth of stromal fibroblasts as well. Clonal growth could be quantitatively determined using an automatic colony counter and it was found that maximal growth response was obtained with 0.5 ng EGF per ml. Using a modified MCDB 151 medium, originally designed for human keratinocytes, clonal growth in low serum and EGF was further improved. The present results thus show that EGF is a potent growth factor for sheep thyroid cells in primary and in clonal culture. Since EGF is a physiologically occurring plasma hormone, it is suggested that it may also be involved in thyroid growth in vivo. (Author abstract) (33 Refs)

4. MODULATION OF PROTEIN PHOSPHORYLATION IN HUMAN COLON ADENOCARCINOMA CELL MEMBRANE PREPARATIONS BY EPIDERMAL GROWTH FACTOR IN VITRO.

Zenisek SC, Fernandez-Pol JA
(c/o Fernandez-Pol) Nuclear Medicine Lab., Veterans Admin Medical Center and Dept. Medicine, Saint Louis Univ., St. Louis, MO, 63125
Int J Cancer; 29(3):277-282 1982

Incubation of membranes prepared from the human colon adenocarcinoma cell line LoVo in vitro with [gamma-32P]ATP demonstrated numerous components whose phosphorylation was stimulated several fold by epidermal growth factor (EGF). One major component of Mn 170 K, which was either undetectable or very weakly phosphorylated in the absence of EGF, was primarily affected by exposure to EGF. The phosphorylation of the 170 K Mr membrane component required the presence of Mn+; Mg+ was ineffective. Although the phosphorylation of many LoVo membrane components was significantly modified by cAMP or dibutyryl-cAMP, none of these cyclic nucleotides appeared to be involved in the phosphorylation of the 170 K membrane component in the presence or absence of EGF. The phosphorylation system of the LoVo membranes efficiently uti lized [gamma-32P]GTP in both the basal and EGF-stimulated reactions. All the membrane components phosphorylated in the absence or presence of EGF, except a band comigrating with the tracking dye front, were digested by trypsin. The possible glycoprotein nature of the 170 K dalton phosphoprotein was indicated by the fact that the 170 K dalton band comigrated with a periodic acid-Schiff base-stained band. These findings suggest that one of the biochemical steps in the mechanism of action of EGF in LoVo cells is enhanced phosphorylation of several membrane proteins, especially that of a glycoprotein of Mr 170 K, by a membrane-bound cyclic nucleotide independent protein kinase. (Author abstract) (23 Refs)

5. AN ASSAY MEASURING THE STIMULATION OF SEVERAL TYPES OF BOVINE ENDOTHELIAL CELLS BY GROWTH FACTOR(S) DERIVED FROM CULTURED HUMAN TUMOR CELLS.

Olander JV, Marasa JC, Kimes RC, Johnston GM, Feder J
Monsanto Co., 800 N. Lindbergh Blvd., St. Louis, MO, 63166
In Vitro; 18(2):99-107 1982

Endothelial cell growth factor(s) from several previously untested human tumor cell lines (ie, SK-HEP-1, MG63, A375, TE671-C1, RD) were detected using a low cell inoculum growth assay. The final cell density in the 2-cm2 wells was determined by a highly sensitive DNA content measurement performed directly in the tissue culture plates. The sensitivity of the assay to human tumor cell growth factors depended critically on the low cell inocula, 2,000 to 5,000 cells/well. Most of the bovine endothelial cells used were cloned from primary cultures; all the cell lines obtained from various fetal and nonfetal sources responded to the growth factor(s) (up to 16x stimulation) as well as to endothelial cell growth supplement. Dose response curves showing the cell specific response of bovine endothelial cells were obtained. The growth stimulatory activity and the in vivo chick embryo chorioallantoic membrane assay responses correlated sufficiently to imply that the assay is detecting tumor angiogenesis factor or some closely related activity. This in vitro assay should prove useful in the identification and purification of tumor-derived factors and in the elucidation of the role of these factors in the events comprising angiogenesis. (Author abstract) (33 Refs)

6. IDENTIFICATION OF A T CELL-DERIVED B CELL GROWTH FACTOR DISTINCT FROM INTERLEUKIN 2.

Howard M, Farrar J, Hilfiker M, Johnson B, Takatsu K, Hamaoka T, Paul WE
Lab. Immunology, Natl. Inst. Allergy and Infectious Diseases, NIH, Bethesda, MD, 20205
J Exp Med; 155(3):914-923 1982

Long-term culture of BALB/c mouse B lymphocytes in which induced supernatants from a T cell hybridoma (B151K12) sustain slow proliferation of lipopolysaccharide-activated B cells suggested the existence of a T cell-derived growth factor. Such a factor was identified in induced supernatants from mouse thymoma EL4 by gel filtration analysis. The B cell growth factor is distinct from interleukin 2. It co-stimulates with anti-IgM antibodies in short term cultures of purified B lymphocytes to produce polyclonal B cell proliferation but not antibody-forming cell production. The factor is not mitogenic for resting B cells and interacts with anti-IgM-activated B cells in a non-H-2-restricted manner. (28 Refs)

7. TWO DISTINCT HUMAN CLONED T CELL LINES THAT EXHIBIT NATURAL KILLER-LIKE AND ANTI-HUMAN EFFECTOR ACTIVITIES.

Sugamura K, Tanaka Y, Hinuma Y
Inst. Virus Res., Kyoto Univ., Sakyo-ku, Kyoto 606, Japan
J Immunol; 128(4):1749-1752 1982

Cloned T cell lines from mixed lymphocyte cultures stimulated with autologous Epstein Barr virus- (EBV) transformed lymphoblastoid cell line (LCL) cells were established using a limiting dilution technique in the presence of T cell growth factor (TCGF). The T cell lines included two distinct clones of cytotoxicity T cells (Tc) in addition to EBV-specific Tc. A cytotoxic profile of one cloned line was similar to that of endogenous NK cells in peripheral blood. The other cloned Tc line showed an anti-human cytotoxicity. The susceptible targets for this latter Tc line were various human cells, including autologous LCL and peripheral blood lymphocytes (PBL), stimulated with pokeweed mitogen, along with NK-sensitive and NK-resistant cell lines. Weak cytotoxic activity was detected against various xenogeneic cell lines. Furthermore, autologous and allogeneic cloned T cell lines were resistant to killing by the anti-human effector clone. These two distinct cloned Tc lines expressed the Leu-1 and Leu-2a antigens, which are markers of suppressor/cytotoxic T cells. (Author abstract) (23 Refs)

8. INDUCTION OF PROLIFERATION AND IG PRODUCTION IN HUMAN B LEUKEMIC CELLS BY ANTI-IMMUNOGLOBULINS AND T CELL FACTORS.

Yoshizaki K, Nakagawa T, Kaieda T, Muraguchi A, Yamamura Y, Kishimoto T
Third Dept. Medicine, Osaka Univ. Medical Sch., Fukushima-ku, Osaka 553, Japan
J Immunol; 128(3):1296-1301 1982

The proliferation and differentiation of human leukemic B cells (B-CLL cells) with anti-Ig and T cell-derived helper factors are described. Stimulation of B-CLL cells with anti-Ig and T helper factors could induce proliferation as well as differentiation into IgM- and IgG-producing cells. Neither anti-Ig nor T helper factors alone could induce any proliferation and/or differentiation of B-CLL cells. Not only whole molecules of anti-Ig but also F(ab')2 fragments could induce proliferation and differentiation of B-CLL cells in the presence of T helper factors, but monovalent Fab' fragments were not effective. Induction of both IgM and IgG with the same idiotype was confirmed by immunofluorescent and SDS-PAGE analysis. By employing an IL 2-dependent cytotoxic T cell line and a TRF-responsive B cell line, T cell factors were separated into a fraction with IL 2 activity but no TRF activity and a fraction with TRF activity but no IL 2 activity by chromatofocusing. Anti-Ig and IL 2 fraction could induce proliferation of B-CLL cells, but TRF fraction was not effective for the induction of proliferation in anti-Ig-stimulated cells. For IgM and IgG production, anti-Ig and both IL 2 and TRF fractions were required. Depletion of IL 2 fraction in the first 2 days' culture inhibited Ig production, whereas the absence of TRF fraction in the first 2 days did not show any inhibitory effect on Ig production. (Author abstract) (26 Refs)

9. HUMAN HELPER T CELL FACTOR(S) (THF). II. INDUCTION OF IGG PRODUCTION IN B LYMPHOBLASTOID CELL LINES AND IDENTIFICATION OF T CELL-REPLACING FACTOR-(TRF)LIKE FACTOR(S).

Teranishi T, Hirano T, Arima N, Onoue K
Dept. Immunobiochemistry, Kumamoto Univ. Sch. Medicine, 2-2-1, Honjo, Kumamoto 860, Japan
J Immunol; 128(4):1903-1908 1982

IgG-PFC was induced in Epstein-Barr virus-transformed B lymphoblastoid cell lines (LCL) by the addition of allogeneic T cells. T cells involved in the induction of IgG-PFC were shown to belong to the Leu 3a+/2a- T cell subset. Furthermore, partially purified soluble factors obtained from the culture supernatant of PPD-stimulated pleural T cells or PWM-stimulated tonsillar mononuclear cells was shown to induce IgG-PFC in LCL across the major histocompatibility complex barrier. The induction of IgG-PFC was observed only in surface IgG-positive LCL cell populations and was not accompanied by the increase in the number of LCL cells. The factors

with such a TRF-like activity were found in two fractions corresponding to the mol wt range of 18,000 to 25,000 (22K fraction) and 28,000 to 38,000 (36K fraction) by gel filtration. Isoelectric focusing at these fractions revealed that TRF-like activity of both 22K and 36K fractions distributed in the pI range of 5.0 to 6.0, and both fractions were found to be devoid of TCGF activity. These results appear to indicate that the factors act on the B cells in terminal stages to trigger final differentiation to Ig-producing cells. (Author abstract) (35 Refs)

10. THE EFFECTS OF T-CELL GROWTH FACTOR AND VIRUS PURIFICATION ON VIRUS-MEDIATED INHIBITION OF LYMPHOCYTE MITOGENESIS.

Wainberg MA, Margolese RG

Lady Davis Inst. Medical Res., Sir Mortimer B. Davis - Jewish General Hosp., 3755 Cote St. Catherine Road, Montreal, Quebec H3T 1E2, Canada

Clin Exp Immunol; 48(1):163-170 1982

Many different types of virus particles are able to non-specifically impede the ability of human peripheral blood lymphocytes to respond to mitogenic or alloantigenic stimuli. This result is not obtained if ultra-purified virus is employed, although virus which has been banded only once through sucrose generally retains inhibitory potential. Ultra-pure virus is relatively unable to bind to cell surfaces, suggesting the importance of physical contact between viruses and cells in order for the observed inhibition to occur. Addition of exogenous T-cell growth factor (TCGF) to cultures containing virus, cells and stimulus causes a dose-dependent reversal of the usual inhibitory effect. (Author abstract) (34 Refs)

11. THE PURIFICATION AND PROPERTIES OF HUMAN T CELL GROWTH FACTOR.

Mier JW, Gallo RC

(c/o Gallo) Lab. Tumor Cell Biology, NCI, NIH, Bethesda, MD, 20205

J Immunol; 128(3):1122-1127 1982

Human T cell growth factor (TCGF) has been purified more than 800-fold from serum-free lymphocyte conditioned media by utilizing ion exchange chromatography with DEAE-Sepharose, gel filtration with Ultrogel AcA54, and preparative SDS-polyacrylamide gel electrophoresis. This mitogenic protein, which is released by T cells into the incubating media after exposure to a lectin (PHA), has a mw of 13,000 as determined by SDS-PAGE and 20,000 to 25,000 on gel filtration and has an isoelectric point of 6.8. The material extracted from acrylamide gels is a single band (or 2 nearly superimposed bands) when rerun on analytical SDS gels. Partially purified material is unstable even at-70 C and requires the addition of bovine serum albumin or polyethylene glycol to maintain biologic activity. It is sensitive to proteolytic digestion but resistant to nucleases and thiol-reducing agents such as dithiothreitol. Human TCGF can be reversibly denatured with urea or SDS. Material extracted from acrylamide gels has been shown to sustain T lymphoblasts in tissue culture. In contrast to lectins, certain antigens, and crude lymphocyte conditioned media, purified TCGF does not initiate lymphocyte blastogenesis but is a highly selective mitogen for T cells previously activated by exposure to lectins or antigens. (Author abstract) (29 Refs)

12. AN ACUTE LYMPHOBLASTIC LEUKEMIA WHICH PRODUCES HUMAN T CELL GROWTH FACTOR.

Anderson DW, Hayward A, Jones C

Eleanor Roosevelt Inst. Cancer Res., Univ. Colorado Health Sciences Center, Denver, CO, 80262

Immunol Commun; 10(8):697-706 1981

We report the production of T cell growth factor activity by a PHA stimulated acute lymphoblastic leukemia cell line designated HSB-2. Factor production was enhanced by the addition of phorbol myristate acetate to the cultures. The growth factor will enhance the proliferation of PHA stimulated thymocytes and sustain the proliferation of growth-factor dependent human T cell blasts. The characteristics of the cell line are described. (Author abstract) (18 Refs)

13. INDUCTION OF NEURAL DIFFERENTIATION BY SERUM DEPRIVATION IN CULTURES OF THE EMBRYONAL CARCINOMA CELL LINE 1003.

Darmon M, Stallcup WB, Pittman QJ

Departement de Biologie Moleculaire, Institut Pasteur, 25 rue du Dr Roux, 75724 Paris Cedex 15, France

Exp Cell Res; 138(1):73-78 1982

When 1,003 embryonal carcinoma cells are passaged from a serum-containing medium to a serum-free medium, the cells continue to grow for approx six generations, during which time they all differentiate. Neuro-epithelial cells are first formed which subsequently differentiate into neurons; a few epithelioid cells also appear. When 1,003 cells are grown to confluency in serum-containing medium a few epithelioid cells appear, but neuro-epithelial and Neuronal derivatives are never found. Immunohistochemical studies utilizing markers of neural tissues have been carried out (1) to identify individual cells and (2) to determine whether induction or selection phenomena were responsible for the morphological changes observed. The nerve-glial antigen NG2 and the nerve-specific antigen N6 were absent in exponential and confluent cultures in serum-containing medium but appeared sequentially in serum-free medium. The NH2 antigen appeared very precociously, but then disappeared from the majority of the cells when they differentiated into neuro-epithelial cells. This antigen, however, was retained on most of the epithelioid cells of these serum-free cultures. The N6 antigen appeared later and was found specifically on pre-neurons and neurons. These results demonstrate that selection - if it occurs - cannot be the mechanism responsible for the appearance of immunologically and morphologically identifiable neural derivatives in serum-free cultures of 1,003. Serum deprivation thus seems to trigger the induction of neural differentiation. (Author abstract) (26 Refs)

14. DIAGNOSIS OF MELANOMA AND NEUROBLASTOMA WITH NERVOUS TISSUE GROWTH FACTOR LABELED WITH 131 IODINE.

Skromne Kadlubik G, Garcia Solis J, Caballero Flores Y, Hidalgo Rico R

Departamento de Fisiologia, Facultad de Medicina de la U.N.A.M., Ciudad Mexico, Mexico, D.F.

Patol Quir Citol Exfoliativa; 7(4):163-165 1981

Nerve growth factor (NGF) labeled with 131I was used in a study of 15 patients with different tumors. The labeled NGF (400 mCi) was injected iv in double blind experiments and was measured scintigraphically 1/2 hr after being injected. Results were positive in 9 patients, 5 of whom had melanoma (ME) and 4 neuroblastoma (NB). Results were negative in six patients with benign tumors such as lipoma and nevus pigmento-

sus. Positive images of the ME and the NB could not be differentiated. Receptors for the NGF were present only in the patients with ME or NB. The 131I compound was well tolerated and nontoxic; the patients were followed up during 1 yr after testing. This test is proposed instead of biopsy for diagnosis of ME and NB. The 131I compound is presently being studied in the therapy of ME and NB. (2 Refs) (In SP A)

15. NOREPINEPHRINE AND NERVE GROWTH FACTOR: SIMILAR PROTEINS PHOSPHORYLATED IN THE NUCLEI OF TARGET CELLS.

Hashimoto S, Guroff G
Lab. Developmental Neurobiology, Natl. Inst. Child Health and Human Development, NIH, Bethesda, MD, 20205
Biochem Biophys Res Commun; 104(4):1477-1483 1982

Treatment of C6 glioma cells (ATCC CCL 107) with the beta-adrenergic agonist isoproterenol, in the presence of (32P) inorganic phosphate, increased radioactivity in two nonhistone nuclear proteins. Comparison of radioautograms of these nonhistone fractions with those of nuclei of superior cervical ganglia treated with nerve growth factor show strong similarity of the proteins. (18 Refs)

16. REDUCTION OF ETHYLNITROSOUREA-INDUCED NEOPLASTIC PROLIFERATION IN RAT TRIGEMINAL NERVES BY NERVE GROWTH FACTOR.

Vinores SA, Koestner A
Inst. Cancer Res., Fox Chase Cancer Center, Philadelphia, PA, 19111
Cancer Res; 42(3):1038-1040 1982

Ethylnitrosourea administered to pregnant rats on the 21st day of gestation at a dose of 50 mg/kg resulted in early neoplastic proliferation or neurinomas of trigeminal nerves in 90% of the offspring at 90 days of age. Treatment with nerve growth factor either postnatally following ethylnitrosourea administration or transplacentally prior to ethylnitrosourea treatment appeared to result in a reduction of neurinomas at 90 days of age. (Author abstract) (17 Refs)

17. NERVE GROWTH FACTOR RESCUES CLONAL PC-12 PHEOCHROMOCYTOMA CELLS FROM 'INOSITOL-LESS DEATH'.

Lakshmanan J, Tarris R, Lee MI, Fisher DA
(c/o Tarris) Perinatal Res. Lab., Dept. Pediatrics, Harbor-UCLA Medical Center, UCLA Sch. Medicine, 100 W. Carson St., Torrance, CA, 90509
Biochem Biophys Res Commun; 105(1):36-43 1982

The growth response of the PC-12 clonal rat pheochromocytoma line cultured over a period of 120 hr was investigated under four conditions: (1) myo-inositol deficient medium (IDM), (2) IDM supplemented with mouse submaxillary gland nerve growth factor (NGF), (3) myo-inositol supplemented medium (ISM), and (4) ISM containing NGF. The PC-12 cells continued to grow un all four conditions for at least 24 hr. By 72 hr, all multiplication ceased under IDM conditions; the cells multiplied most efficiently with ISM or ISM + NGF. An examination of cell viability showed that after 24 hr culture, viability exceeded 98% for all four conditions. After 24 hr, cell viability progressively declined in IDM but was maintained at a steady state in the IDM + NGF, ISN, and ISM + NGF media. The absolute requirement of myo-inositol for growth and viability is apparently dependent on cell density; differential responses were seen at high and low cell densities either in the absence or presence of myo-inositol. Neither scyllo-inositol nor myo-inositol 1,2-cyclic phosphate could substitute for myo-inositol in supporting cell survival or growth. The results show that myo-inositol is required for growth and viability of PC-12 cells and NGF has the ability to rescue the cells from 'inositol-less death' and to promote cell multiplication in inositol-deficient medium. (15 Refs)

18. A GROWTH FACTOR PRODUCED BY WEHI-3 CELLS FOR MURINE HIGH PROLIFERATIVE POTENTIAL GM-PROGENITOR COLONY FORMING CELLS.

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Cell Biol Int Rep; 6(3):243-251 1982

Preparation and properties of 'synergistic activity' (SA), a hemopoietic growth factor, from media conditioned by a myelomonocytic leukemic cell line (WEHI-3) are described. SA was prepared in high yield by conditioning serum-free medium with WEHI-3 cells that had been grown in vivo (BALB/c mice). SA was shown not to be stored in WEHI-3 cells. Storage at 4C and-20 C did not affect the activity. SA was stable between pH 2.0 and 10.6 and to heating for 1 hr at less than or equal to 50 C, but was unstable to storage at room temperature, trypsin digestion, 8 M urea with and without 4 milliM mercaptoethanol (ME) at pH 8.0, and to ME alone. Instability in the presence of ME indicated the presence of a disulfide bond, while inactivation by trypsin suggested that SA is a protein. The apparent mol wt was 9,000-25,000, and the isoelectric point was at pH 4.0-6.5. SA did not bind to concanavalin-A Sepharose. Morphologic studies showed that all colonies contained virtually pure populations of macrophages. (10 Refs)

19. REGULATION OF THE COLONY-STIMULATING ACTIVITY PRODUCED BY A MURINE MARROW-DERIVED CELL LINE (H-1).

Garnett HM, Cronkite EP, Harigaya K
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Proc Natl Acad Sci USA; 79(5):1545-1548 1982

The production of molecular species that stimulate growth of granulocyte or macrophage colonies (GM-CSF) by the fibroblastoid H-1 cell line is unaffected by either native or iron-saturated lactoferrin, although some inhibition is detected with 10 uM prostaglandin E1. The H-1 GM-CSF is able to support the formation of macrophage, neutrophil, and mixed colonies. Feeder layers of H-1 cells are also able to support the development of colony-forming units stimulated by GM-CSF (GM-CFUc) although the number of colonies produced when the optimal H-1 cell concentration is plated (2.5 x 10(3) cells) is only 30% of the number with conditioned medium alone. This inhibitory effect is observed irrespective of the presence of an additional agar layer between the feeder cells and plated bone marrow cells, implying that diffusable substances are involved. Addition of indomethacin (10 uM) to feeder layers derived from 2.5 x 1 0(3) H-1 cells increases the number of GM-CFUc detected to 50% of that seen with conditioned medium alone. This result suggests that released prostaglandin may be responsible for some, but not all, of the observed inhibition of colony formation. In the presence of the H-1 feeder layers, only macrophage colonies are detected and hence it appears that the H-1 cells produce, in addition to prostaglandin, a diffusible inhibitory substance that preferentially inhibits granulopoiesis. (Author abstract) (28 Refs)

20. REGULATION OF THYMIDINE KINASE ENZYME LEVEL IN SERUM-STIMULATED MOUSE 3T6 FIBROBLASTS.

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Dept. Biochemistry, Ohio State Univ., Columbus, OH, 43210
Exp Cell Res; 138(1):79-85 1982

This report describes the regulation of thymidine kinase (TK) enzyme level in mouse 3T6 fibroblasts experiencing a serum-induced transition between the resting (G0) and growing states. The specific activity of TK is about 200-500 pmoles TMP formed x min-1 x mg protein-1 in exponentially growing cells but is scarcely detectable (usually less than 5 pmoles TMP formed x min-1 x mg protein(-1)) in cells resting at confluence in medium containing 0.5% serum. When the resting cells are serum-stimulated to re-enter the cell cycle, TK activity remains at the resting level until about 12 hr following stimulation, but then increases dramatically as the cells enter S phase. Experiments with inhibitors of protein or RNA synthesis indicate that the increase in enzyme level is the result of de novo synthesis of the enzyme, and that the increase depends on transcription. The increase in TK level is not blocked when cells are stimulated in the presence of inhibitors of DNA synthesis, indicating that there is no tight coupling between these two processes. The increase is not blocked by the presence of thymidine ranging in concentration from 10(-7) to 10(-3) M, suggesting that fluctuations in the intracellular concentrations of thymidine or thymidylic acid do not affect the increase in TK level. When cells are treated with cycloheximide, the level of TK decreases with a 4-5 hr half-life, indicating that the enzyme is relatively unstable. When the serum stimulus is withdrawn from cells in S phase (20 hr following stimulation), the TK level decreases with the same 4 hr half-life beginning about 6 hr following serum withdrawal. The ability of the cell to control the rate of production of TK over a very wide range, along with the relative instability of the enzyme appear to be responsible for the rapid and wide variations in TK level during the cell cycle The increase in the level of TK appears to be regulated in a manner similar to that of dihydrofolate reductase and thymidylate synthetase in serum-stimulated 3T6 cells, suggesting that the expression of these genes (and perhaps those for other S phase enzymes) may be coordinated by a common control signal. (Author abstract) (23 Refs)

21. DIFFERENT GROWTH RESPONSES OF HAMSTER DERMAL FIBROBLASTS AND CHONDROCYTES TO PLATELET-DERIVED GROWTH FACTOR IN CELL CULTURE.

Katoh Y, Takayama S
Dept. Experimental Pathology, Cancer Inst., 1-37-1, Kami-Ikebukuro, Toshima-ku, Tokyo 170, Japan
Dev Growth Differ; 23(5):253-531 1981

Growth responses of dermal fibroblasts (DF) and chondrocytes (DC) obtained from 1-wk-old hamsters to platelet-derived growth factor (PDGF) were compared. Results indicate that PDGF is primarily responsible for the growth of DF, but not for that of DC. Partially purified PDGF, added to the medium with 0.5% fetal calf serum, stimulated DNA synthesis in DF, but not in DC. (24 Refs)

22. GROWTH FACTORS ADHERENT TO CELL SUBSTRATE ARE MITOGENICALLY ACTIVE IN SITU.

Smith JC, Singh JP, Lillquist JS, Goon DS, Stiles CD
Mill Hill Laboratories, Imperial Cancer Res. Fund, Burtonhole Lane, London NW7 1AD, England
Nature; 296(5853):154-156 1982

Radiolabeled (125I) platelet derived growth factor (PDGF) and pituitary-derived fibroblast growth factor (FGF) bound rapidly and tenaciously to tissue culture plates coated with either a collagen (a major component of the extracellular matrix) or uncoated. This binding was resistant to extraction with nonionic detergents, organic solvents and chaotropic solvents. When quiescent 3T3 cells were plated out in culture dishes pretreated with PDGF, DNA replication was induced in the absence of any PDGF added to the medium, thus indicating that PDGF bound to the surface is mitogenically active. It is concluded that PDGF and FGF adhere tenaciously to the surface of tissue culture plates and they can stimulate proliferation of cells maintained in platelet-poor plasma. The results may have a bearing on tissue culture analyses of extracellular matrix materials, and their role in controlling cell growth and function, and may be relevant to the well characterized 'anchorage requirement' for fibroblast growth in vitro. (18 Refs)

23. MOUSE EMBRYOS CONTAIN POLYPEPTIDE GROWTH FACTOR(S) CAPABLE OF INDUCING A REVERSIBLE NEOPLASTIC PHENOTYPE IN NONTRANSFORMED CELLS IN CULTURE.

Proper JA, Bjornson CL, Moses HL
Section Experimental Pathology, Dept. Cell Biology, Mayo Clinic/ Foundation, Rochester, MN, 55905
J Cell Physiol; 110(2):169-174 1982

Seventeen-day Swiss albino mouse embryos were subjected to an acid/ethanol extraction which has been used to isolate transforming-growth factors (TGF) from tumors. After dialysis against 1% acetic acid, the extract was lyophilized and clarified. Various concentrations were tested for their ability to stimulate AKR-2B mouse cells to form colonies in soft agar. The extract was effective at concentrations greater than 10 ug/ml. A correlation between protein concentration and enhancement of the size and number of colonies formed was apparent. When fractionated on a Bio-Gel P-60 column, the major peak of colony-stimulating activity (CSA) was in the 15,000 mol wt range. A smaller peak of CSA was observed in the 22,000 mol wt range with the higher concentration of the fractions. Late eluting epidermal growth factor (EGF) activity was observed which probably represents embryonal EGF. Pooled material containing the major peak of CSA produced a 50% level of CSA in the 100 ug range similar to the effect of TGF on transformed AKR cells. At a level of 1 mg/ml, the pooled material caused 50% competition of 125I-EGF binding to A431 human squamous cell carcinoma clone cell EGF receptors, which is also comparable to the activity of TGF from transformed AKR cells. The substance, designated as mouse embryo factor (MEF) caused AKR-2B cells to assume a transformed phenotype identical to that of the chemically transformed AKR cells. Effects of heat indicated that, like TGF, the biological activity of MEF is due to a polypeptide with disulfide bonds. Further separation revealed two peaks of soft agar dermal growth factor receptor-competing activity. The similarities of MEF to previously identified transforming growth factors suggest that both fetal development and neoplastic transformation may be affected by similar mechanisms. (15 Refs)

24. REPLICATION AND PHENOTYPIC EXPRESSION OF CONTROL AND SCLERODERMA HUMAN FIBROBLASTS: RESPONSES TO GROWTH FACTORS.

LeRoy EC, Mercurio S, Sherer GK
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Proc Natl Acad Sci USA; 79(4,part1):1286-1290 1982

To explore the mechanism of increased collagen synthesis by scleroderma skin fibroblasts in vitro, control and scleroderma fibroblasts were compared in confluent monolayer cultures growth-arrested by serum deprivation; responses to optimal mitogenic doses of platelet-derived growth factor, fibroblast growth factor, epidermal growth factor and nerve growth factor were compared. Platelet-derived growth factor had a selective mitogenic effect on control skin fibroblasts not observed with scleroderma skin fibroblasts. None of the factors studied had a selective effect on collagen synthesis independent of cell replication; scleroderma and control fibroblasts responded similarly. Therefore, the growth factors studied may not be involved in generating the activated scleroderma fibroblast directly; platelet-derived growth factor may play an indirect role in fibroblast replication in human fibrotic disorders. (Author abstract) (30 Refs)

25. INFLUENCE OF LOCAL FACTORS ON THE GROWTH OF TUMORS.

Taradi M
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Lijec Vjesn; 103(4/5):212-213 1981

Influence of local factors on tumor growth is discussed. Intracellular reactions have an important role in growth regulation, and changes in intercellular communications could cause or express the neoplastic process. Chalones, angiogenesis, and nonspecific macrophage reactions are also important factors of the microenvironment influencing the tumor growth. (30 Refs) (In SCR)

26. ROLE OF CHALONES IN THERAPY OF LEUKEMIAS.

Bala IuM, Kovalevskaia NP
Central Res. Lab., Medical Inst., Voronezh, USSR
Probl Gematol Pereliv Krovi; 26(12):12-16 1981

The therapeutic efficacy of granulocytic chalones was studied in vitro in cultured cells of experimental leukemias and in vivo in 36 patients with myeloid leukemia. The in vitro experiments showed that the chalones inhibited the proliferative activity in cells of myeloid Graffi leukemia but failed to inhibit proliferative activity in cells of lymphoid and nondifferentiated leukemias La, P-388, and L-1210. The in vivo experiments showed that the chalones reduced proliferative activity in bone marrow cells of the patients with acute and chronic myeloid leukemia, although the sensitivity of blast cells to chalones was 5-7x lower than that of normal granulocytes. Blood levels of granulocytic chalones in the patients with myeloid leukemia were 5-20x greater than that in healthy controls. The most pronounced increase in chalone level was observed in the chronic myeloid leukemia patients who had leukocytosis and hepatomegaly. Effective chemotherapy resulted in normalization of chalone levels. (12 Refs) (In RUS)

HORMONES/ STEROIDS/ PROSTAGLANDINS AS GROWTH EFFECTORS

27. SITE-SELECTIVE GROWTH OF A HORMONE-RESPONSIVE HUMAN BREAST CARCINOMA IN ATHYMIC MICE.

White AC, Levy JA, McGrath CM
Dept. Tumor Biology, Michigan Cancer Foundation, Detroit, MI, 48201
Cancer Res; 42(3):906-912 1982

MCF-7 is a human breast cancer line which requires estradiol supplementation for growth in sc tissues of athymic mice. In order to evaluate the influence of host site on hormone-dependent tumorigenicity and growth, MCF-7 cells were inoculated into tissues varying in ability to concentrate exogenous estradiol. Tumorigenicity was defined in terms of latency, threshold inoculum size, and tumor growth and progression. We observed that sites such as lung rarely supported MCF-7 tumors. However, moderately estrophilic sites such as mammary fat and adjacent subcutaneum and dermis supported the growth of small MCF-7 tumors from large tumor cell inocula, but only in estrogenized mice. In contrast, the highly estrophilic sites, brain and periuterine tissues, produced rapidly growing tumors from small cell inocula. Only in periuterine tissues did tumors develop in the absence of exogenous estradiol. These studies demonstrate that tu morigenicity and growth rates of estrogen-dependent MCF-7 tumors vary as a function of tissue implantation site. (Author abstract) (46 Refs)

28. INTERACTION OF PROLACTIN, ESTROGEN AND PROGESTERONE IN A HUMAN MAMMARY CARCINOMA CELL LINE, CAMA-1. I. CELL GROWTH AND THYMIDINE UPTAKE.

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J Steroid Biochem; 15:421-427 1981

The growth and thymidine (TDR) uptake of a human mammary carcinoma cell line (CAMA-1) in long term monolayer culture were investigated. The cells were maintained in the log phase for 7 days in Eagles' minimum essential medium with Earle's salt, supplemented with 15 or 25% fetal bovine serum (FBS) without further feeding. The effects of FBS, dextran-charcoal treated FBS (DCFBS), and serum substitute (SS) were investigated. CAMA-1 cells grew poorly in SS as compared to 25% FBS. With addition of either 1% FBS or DCFBS cell proliferation in SS substantially improved. Ten percent SS plus 10% DCFBS yielded fewer cells on day 7 than conditions with SS plus 1% serum. Apparently, FBS must also contain certain inhibitors of cell growth. When cells were grown in the absence of added estradiol (ED) in DCFBS, cell growth was substantially reduced. Effect of ED is dose-dependent, 0.1 milliM inhibited and 10 nanoM stimulated cell growth. Estrogen (ES) not only stimulated cell growth, it also stimulated the uptake of TDR into cells. Prolactin (PR) by itself, or even in the presence of ES, had no effect upon cell proliferation. The effect of combined treatment with ES and PR on TDR uptake into cells was examined in 10% DCFBS and 10% SS plus 1% DCFBS. ED in absence of PR affected an approx 1.5-fold increase of TDR uptake; with SS, no ED-induced TDR uptake was exhibited. A dichotomous effect of progesterone (PG) upon TDR was observed, low levels of PG had only slight stimulatory effect and had additional stimulation upon ES-induced TDR uptake. PG at 10 uM uniformly in-

hibited TDR uptake either in the presence or absence of ED. Results indicate that the CAMA-1 cell line is a good alternative model for the investigation of hormonal interaction. (23 Refs)

29. HORMONAL REGULATION OF NET DNA SYNTHESIS IN MCF-7 HUMAN BREAST CANCER CELLS IN TISSUE CULTURE.

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Cancer Res; 42(5):1727-1735 1982

Estrogens and anti-estrogens have striking effects on growth of target tissues in vivo. In order to examine these effects in vitro, experimental methods for the rigorous assessment of rates of net DNA synthesis in tissue culture systems are required. The quantitation of DNA synthesis with inorganic [32P]orthophosphate is described. The limitations of more conventional [3H]thymidine labeling are illus trated in this hormonally responsive system. Estrogen administration increases the specific activity of acid-soluble phosphate and increases net DNA synthesis relative to controls in MCF-7 human breast cancer cells. Stimulation is most evident after 36 hr of hormone treatment. Conversely, tamoxifen produces substantial inhibition of net DNA synthesis after 36 hr of hormone treatment. Thymidine availability modulates the effect of estrogens and anti-estrogens on DNA synthesis and constitutes an independent experimental variable. (Author abstract) (55 Refs)

30. ROLE OF ESTROGENS AS PROMOTERS OF HEPATIC NEOPLASIA.

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Dept. Pathology, Toronto Western Hosp., Toronto, Ontario, Canada
Lab Invest; 46(3):313-320 1982

The administration of estrogens to humans has been associated with the development of benign and possibly malignant hepatocellular neoplasms. This study was designed to investigate the mechanism of this association. We present a rat model that demonstrates that stilbestrol (DES) and ethinyl estradiol promote the development of hepatic neoplasms in rats previously initiated with diethylnitrosamine (DEN). Eighty male and 12 female Fischer-344 rats were given a single intraperitoneal injection of either DEN (200 mg/kg of body wt) or saline. Beginning 4 wk after injection, the rats were given an estrogen or corn oil twice weekly for up to 50 wk. Treatments were stopped at the time of sacrifice or 11 to 29 wk prior to sacrifice. Estrogen treatments included high dose DES (5 mg per dose), low dose DES (0.5 mg per dose), and ethinyl estradiol (0.2 mg per dose). Male and female rats given both DEN and high dose of DES developed grossly visible hepatic hyperplasic nodules (mean, 9.1 per liver) after 20 wk of DES. Nodules also developed if the onset of DES treatment was delayed until 28 wk after initiation. Rats given DEN alone or DES alone did not develop nodules after 20 wk. Microscopic hyperplastic foci also developed in rats given DEN plus DES, DES alone, and DEN alone. The foci in rats given DES alone were largely reversible on cessation of estrogen therapy. Mitotic activity in foci and nodules was prominent during estrogen therapy but declined markedly after cessation of therapy. Similar promoting activity of ethinyl estradiol was observed. Low dose DES was not effective in promoting tumor formation. The data indicate that estrogens promote hepatic neoplasia, perhaps by increasing hepatocyte mitotic activity and thereby facilitating the evolution of previously initiated cells into neoplastic clones. Comparison with human disease should be made with caution, especially because the estrogenic dose administered was approx 200-fold the usual contraceptive dose in humans. However, the analogy between this model and the development of human oral contraceptive-associated hepatic tumors is evident. It is possible that some women have latent 'initiated' cells that divide faster than the surrounding hepatocytes in response to estrogens. (Author abstract) (29 Refs)

31. BEHAVIOR OF PRIMARY AND SERIALLY TRANSPLANTED ESTROGEN-DEPENDENT RENAL CARCINOMA CELLS IN MONOLAYER AND IN COLLAGEN GEL CULTURE.

Talley DJ, Roy WA, Li JJ
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In Vitro; 18(2):149-156 1982

When estrogen is present in culture medium, enzyme-dissociated cells from estrogen-induced primary renal tumors in Syrian hamsters and from 1st to 4th serially transplanted carcinomas in monolayer culture contain progesterone receptor levels that are similar and comparable to those in tumors in vivo (ie, 2 pmol/3 x 10(6) cells). Despite the similarity of receptor levels in cultured cells isolated from primary and transplanted tumors, the ability of cells to be maintained in culture differs considerably from one tumor stage to another. When cultured as monolayers in plastic flasks, isolated cells from primary tumors exhibit a marked decline in cell number after 4 to 6 days in culture. On the other hand, monolayer-cultured cells from first and second transplantation tumors remain essentially constant in cell number over a 2 wk culture period and cells from third transplantation tumors undergo a two- to threefold increase in cell number during 2 wk in culture. When primary tumor cells are cultured in collagen gels, the decline in cell number over a 2 wk culture period is prevented and progesterone receptor levels remain elevated. Cells cultured from first transplantation tumors exhibit a delayed decline in cell number beginning after 2 wk in monolayer culture. The decline in cell number in monolayer culture, like that for cells from primary tumors, can be prevented by culturing cells from first transplantation tumors in collagen gels. Neither cells from primary nor first transplantation tumors exhibit significant increases in cell number in collagen gels. Increasing the serum concentration of growth medium to 30% does not stimulate growth of cells under these conditions. Cells isolated from fourth transplantation tumors undergo a fourfold increase in cell number over a 1 mo culture period whether cells are cultured as monolayers or in collagen gels. (Author abstract) (23 Refs)

32. AN ANALYTIC CELLULAR EVALUATION OF ASPIRATED SMEARS FROM TRANSPLANTED HUMAN ENDOMETRIAL ADENOCARCINOMA IN NUDE MICE TREATED WITH 17BETA-ESTRADIOL AND PROGESTERONE.

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Acta Cytol (Baltimore); 26(2):251-258 1982

The effects of 17beta-estradiol and progesterone on human endometrial adenocarci nomas grafted into BALB/c nude mice were studied. Cytologic studies were made on aspiration biopsy smears obtained from the grafts with a fine needle. Mitotic figures were frequently observed in the cells obtained from 17beta-estradiol-treated mice, whereas there were few mitotic figures in cells from progesterone-treated ones. Progester-

one produced such morphologic changes as multinucleolation and cytoplasmic vacuolization. Untreated tumors and those treated with 17beta-estradiol were solid and were interpreted as poorly differentiated adenocarcinomas. Progesterone-treated tumors were slightly soft but were also interpreted as poorly differentiated adenocarcinomas; however, central necrosis was seen frequently, and differentiation occurred at the margin of these tumors. 17beta-estradiol promoted increased tumor growth, whereas progesterone produced differentiation and inhibited tumor growth. (Author abstract) (18 Refs)

33. INHIBITION BY 17BETA-ESTRADIOL OF THE GROWTH OF THE RAT PITUITARY TRANSPLANTABLE TUMOR MTF4.

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Cancer Res; 42(4):1492-1497 1982

The effects of pharmacological doses of estradiol on the growth of the rat pituitary transplantable tumor MtF4 have been characterized. After sc injection into control rats of a cell suspension prepared from the tumor, in 12 experiments, the av range of days when a new tumor became palpable was between 14.7 +-2.3 (SD) and 24.5 +-3.3. rats. Daily im injections of 10 ug 17beta-estradiol benzoate or 17beta-estradiol implanted in Silastic tubing partially inhibited the tumor growth as judged by the wt and the time of appearance of the tumor. The same treatments increased the pituitary wt. Both effects were dose dependent above 3 ug 17beta-estradiol benzoate per day and displayed some specificity. 17alpha-Estradiol did not modify the pituitary or the tumor growth significantly; 17beta-hydroxy-3-keto-5alpha-androstane also did not modify the tumor growth. The inhibitory effect of 17beta-estradiol on tumor growth has also been observed in hypophysectomized rats. The tumor cytosol contained 30 to 220 fmol of specific estrogen-binding sites per mg protein which were characterized by: a high affinity (Kd, 0.25 to 0.65 x 10-9M); a sedimentation constant of 6 to 8S; and their hormone specificity. Thus, 17alpha-estradiol, which was inactive in vivo, had an affinity only four-fold lower than 17beta-estradiol. We conclude that pharmacological doses of 17beta-estradiol inhibit in a dose-dependent manner and with some specificity the growth of the MtF4 transplantable pituitary tumor while increasing the growth of pituitary. We suggest this inhibition requires estrogen receptors and is not mediated via the pituitary, but we cannot specify whether this inhibition wa s direct or not. (31 Refs)

34. GROWTH INHIBITORY EFFECTS OF THYROID HORMONES ON ANDROGEN-DEPENDENT MAMMARY TUMOR CELLS.

Natoli C, Sica G, Natoli V, Scambia G, Iacobelli S
Laboratorio Endocrinologia Molecolare, Universita Cattolica del Sacro Cuore, Via della Pineta Sacchetti 644, 00168 Roma, Italy
J Steroid Biochem; 15:409-413 1981

Proliferative effects of thyroid hormones and their possible reaction with androgens were studied in Shionogi 115 mouse mammary carcinoma. Cells derived from this tumor are available in culture as androgen responsive (+A cells) variant, whose growth is stimulated by physiological concentrations of androgens, and as the autonomuos variant (-A cells). Presence of triiodothyronine receptors in both +A and -A cells was also investigated. Correspondence between binding capacity of the nuclear receptor and biological activity of various thyroid hormone analogues suggests that the proliferative effect is mediated via a receptor mechanism. (23 Refs)

35. SUBHYPOTHALAMIC GRAFTS OF HUMAN PITUITARY ADENOMAS IN TOTAL-BODY IRRADIATED RATS.

Landolt AM, Barker M, Deen DF, Wilson CB
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Cell Tissue Res; 221(2):269-277 1981

Human pituitary adenomas proliferate neither in cell culture nor in athymic 'nude' mice. We propose that one or several of the humoral factors necessary for the growth of pituitary adenomas is missing in these experimental environments. The purpose of our experiments was to examine the possible influence of the hypothalamus in supporting cellular proliferation, and thus adenoma growth. Fragments from four human pituitary adenomas (three pituitary prolactinomas; one ACTH-secreting adenoma) were transplanted into the pituitary fossa of total-body irradiated, hypophysectomized rats. The rats were killed after 2 wk and perfused with a mixture of formalin and India ink. Histologic examination of serial sagittal sections through the pituitary fossa and the adjacent brain showed: vascularization of the grafts from the pituitary stalk and from the scar tissue in the sphenoid bone; survival of some adenomas; and numerous mitoses in an ACTH-secreting specimen obtained from a patient who had Cushing's disease. We conclude from these experiments that as yet unidentified hypothalamic factors are essential for the growth of certain types of pituitary adenomas. (Author abstract) (21 Refs)

36. PROSTAGLANDIN E2-MEDIATED ENHANCEMENT OF HUMAN PLASMA CELL DIFFERENTIATION.

Lydyard PM, Brostoff J, Hudspith BN, Parry H
Dept. Immunology, Middlesex Hosp. Medical Sch., London W1, England
Immunol Lett; 4(3):113-116 1982

Addition of prostaglandin E2 (PGE2) to cultures of lymphocytes from normal subjects enhanced plasma cell differentiation in the presence of pokeweed mitogen (PWM). T-cell mitogenesis to concanavalin A was inhibited with the same concentrations of PGE2. PGE2 failed to enhance plasma cell (PC) differentiation of lymphocytes from patients with systemic lupus erythematosus. Indomethacin in the presence of PWM suppressed PC differentiation in 2/4 experiments. (23 Refs)

37. MULTIPLICATION-STIMULATING ACTIVITY STIMULATES THE MULTIPLICATION OF F9 EMBRYONAL CARCINOMA CELLS.

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Endocrinology; 110(4):1231-1237 1982

The present study was designed to assess the effectiveness of multiplication-stimulating activity (MSA), an insulin-like growth factor, in supporting the growth of F9 cells (an embryonal carcinoma line with properties similar to embryonic stem cells). Under serum-free growth conditions in a medium supplemented with transferrin and fibronectin, MSA is an effective mitogen. At 100 ng/ml, MSA completely replaces the growth requirement for fetal calf serum. Biologically active MSA polypeptides II and III act as potent growth promoters of F9 cells, whereas MSA I appears to be somewhat less effec-

tive. Binding studies carried out with [125I]iodo-MSA indicate that F9 cells possess specific receptors for MSA. Scatchard analysis indicates a single class of MSA receptors with a Ka of 8.2 x 10(9) M-1; about 55,000 MSA molecules can bind per F9 cell. Insulin-like growth factors I and II both compete for MSA binding, whereas insulin does not compete. Since insulin is an effective promoter of F9 cell growth, these results indicate that the mitogenic action of insulin toward F9 cells is not mediated through MSA receptors. It is apparent from these results that MSA is capable of serving as an early embryonic growth factor. (Author abstract) (35 Refs)

CYCLIC NUCLEOTIDE/ POLYAMINES/ NUCLEOTIDES AS GROWTH EFFECTORS

38. ROLE OF POLYAMINES IN EXPRESSION OF THE DIFFERENTIATED PHENOTYPE OF CHONDROCYTES IN CULTURE.

Takano T, Takigawa M, Suzuki F
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Med Biol; 59(5/6):423-427 1981

The role of putrescine, spermidine, and spermine in the expression of the differentiated phenotype of rabbit costal chondrocytes in culture was studied. When added to chondrocytes in the logarithmic growth phase, the polyamines stimulated expression of the differentiated phenotype of the chondrocytes without affecting cell proliferation. The optimal concentration for stimulation was 10(-7) to 10(-6) M. (27 Refs)

39. ORNITHINE DECARBOXYLASE: ESSENTIAL IN PROLIFERATION BUT NOT DIFFERENTIATION OF HUMAN PROMYELOCYTIC LEUKEMIA CELLS.

Luk GD, Civin CI, Weissman RM, Baylin SB
Oncology Center, Johns Hopkins Univ. Sch. Medicine, Baltimore, MD, 21205
Science; 216(4541):75-77 1982

The ornithine decarboxylase inhibitor DL-alpha-difluoromethyl ornithine inhibited a proliferation-associated increase in ornithine decarboxylase activity in cultured human promyelocytic leukemia cells, resulting in a marked suppression of cell proliferation and subsequent cell loss. It also inhibited increases in ornithine decarboxylase activity associated with the phorbol ester-induced conversion of promyelocytic HL-60 cells to monocyte-like cells and the retinoic acid-induced conversion to granulocyte-like cells. However, the inhibition of ornithine decarboxylase activity did not prevent cellular differentiation. These results suggest that polyamine biosynthesis has a specific role in cell proliferation rather than in inducing differentiation that is not accompanied by proliferation. The data also demonstrate that cessation of proliferation in HL-60 cells is not necessarily associated with differentiation. (Author abstract) (18 Refs)

40. ROLE OF POLYAMINES IN HELA CELL PROLIFERATION.

Herbst EJ, Elliot QD
Dept. Biochemistry, Univ. New Hampshire, Durham, NH
Med Biol; 59(5/6):410-416 1981

Nuclei isolated from HeLa cells by the method of Krokan were severely depleted of polyamines by administration of alpha-difluoromethylornithine (DFMO), the irreversible inhibitor of ornithine decarboxylase. Simultaneously, cells were synchronized for S phase DNA synthesis by a double thymidine block procedure. The inhibitor of S phase DNA synthesis in nuclei isolated from cells synchronized in media containing DFMO appears to be caused by depletion of polyamines in these cells. Replenishment of intracellular polyamines, especially spermidine, by administration of exogenous putrescine, spermidine, or spermine overcomes the DNA replication deficiency. The effect of polyamines is evidently indirect; a role in RNA polymerase I activity is possible. (29 Refs)

COFACTORS/ NUTRITIONAL GROWTH FACTORS

41. THE EFFECT OF ALTERED LUMINAL NUTRITION ON CELLULAR PROLIFERATION AND PLASMA CONCENTRATIONS OF ENTEROGLUCAGON AND GASTRIN AFTER SMALL BOWEL RESECTION IN THE RAT.

Sagor GR, Al-Mukhtar MY, Ghatei MA, Wright NA, Bloom SR
(c/o Bloom) Dept. Medicine, Royal Postgraduate Medical Sch., London, England
Br J Surg; 69(1):14-18 1982

Luminal nutrition is known to have a trophic effect on small bowel mucosa after intestinal resection. Humoral agents, however, may also contribute to this process. Two of the proposed humoral agents, enteroglucagon and gastrin, were therefore investigated after intestinal resection and transection in the rat, and changes in their concentration in the plasma were related to cellular proliferation. Forty-eight male Wistar rats had either 75% proximal small bowel resection or jejunal transection. The animals we re further divided into three groups, each with a different nutritional intake. The first group were allowed food ad libitum. The second group were kept under hypothermic conditions which resulted in hyperphagia, while the last group were nourished intravenously. A further eight animals had a laparotomy only (sham operation). All animals were killed 12 days after operation, plasma enteroglucagon and gastrin were measured, while determination of the crypt cell production rate was used to denote cellular proliferation. In each group resected rats had significantly higher crypt cell production rates and greater enteroglucagon levels compared with transected animals. However, only in the normally fed group was plasma gastrin increased in resected animals, there being no significant difference in the plasma concentration of this peptide in transected compared with resected rats, in both the intravenously fed and hyperphagic groups. In the models studied enteroglucagon appears to be a more likely candidate for a humoral trophic agent than gastrin in intestinal adaptation. (Author abstract) (41 Refs)

42. DECREASED SYNTHESIS OF LARGE FUCOSYL GLYCOPEPTIDES DURING DIFFERENTIATION OF EMBRYONAL CARCINOMA CELLS INDUCED BY RETINOIC ACID AND DIBUTYRYL CYCLIC AMP.

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Dev Biol; 90(2):441-444 1982

The F9 clone of a teratocarcinoma stem cell line (EC) was induced to differentiate in vitro by retinoic acid (1 uM) and dibutyryl cyclic AMP (1 milliM). Synthesis of large fucosyl glycopeptides (belonging to the lactosaminoglycan series, with a galactose and N-acetylglucosamine core structure) declined as the F9 cells differentiated morphologically. The amount of fucose in the particulate fraction of cells (calculated from the protein content) declined slightly during cell differentiation. The differentiation system of F9 cells, with a clear-cut change in the high-mol wt carbohydrate profile during differentiation, is a suitable model for further study of the causal relationship between altered glycosylation and induction of cell differentiation. (14 Refs)

43. RETINOIC ACID INDUCES ANCHORAGE- AND DENSITY-DEPENDENT GROWTH WITHOUT RESTORING NORMAL CYTOSKELETON, EGF BINDING, FIBRONECTIN CONTENT AND ODC ACTIVITY IN A RETROVIRUS-TRANSFORMED MOUSE CELL LINE.

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Exp Cell Res; 138(1):95-107 1982

We studied the effects of retinoic acid (RA), a known tumor inhibitor, on a group of transformation-related properties expressed by a Kirsten sarcoma virus (Ki-MSV)-transformed BALB/c 3T3 (clone A31) cell line (KA31). The aim was to determine whether any one or a group of such transformation-speci fic changes, exhibited by this cell line, is an absolute prerequisite for its acquisition of the neoplastic state. Our study shows that RA treatment restores two important parameters of normalcy in KA31 cells: density-dependent growth inhibition, and anchorage-dependent growth. Retinoic acid also induces cell-to-substratum adhesiveness in a significant proportion of the treated KA31 cell population. However, four other markers of transformation - loss of microfilaments, lack of surface fibronectin, reduced epidermal growth factor (EGF) binding, and increased ornithine decarboxylase activity - remain unaltered by RA treatment. These transformation-specific changes, therefore, are dissociable from loss of anchorage- and density-dependent growth. It is suggested that RA may intervene in transforming protein kinase-induced cell surface changes that are involved in cellular malignancy and the loss of growth control in KA31 cells. (Author abstract) (57 Refs)

44. RETINOIC ACID ENHANCES COLONY-STIMULATING FACTOR-INDUCED CLONAL GROWTH OF NORMAL HUMAN MYELOID PROGENITOR CELLS IN VITRO.

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Div. Hematology/Oncology, Dept. Medicine, Univ. California Los Angeles, Sch. Medicine, Los Angeles, CA, 90024
Exp Cell Res; 138(1):193-198 1982

We studied the effect of vitamin A and its analogues (retinoids) on the clonal growth in vitro of normal human myeloid progenitor cells. Normal human bone marrow cells were cultured in soft gel in the presence of a source of colony-stimulating factor (CSF), and various retinoids, and the number of granulocyte-macrophage colonies (CFU-GM) were scored. The addition of 3 x 10(-8) to 3 x 10(-6) M retinoic acid to culture plates containing SCF markedly increased the number of myeloid colonies as compared with culture plates containing CSF alone. Maximal stimulation occurred at a concentration of 3 x 10(-7) M retinoic acid which increased the mean number of colonies by 213 +-8% (+- SE) over plates containing CSF alone. Retinal or retinyl acetate was less potent than retinoic acid, and retinol (vitamin A) had no effect on colony growth. Retinoic acid had no direct CSF activity nor did it stimulate CSF production by the cultured bone marrow cells. Our studies show for the first time that retinoids can stimulate granulopoiesis in vitro and we suggest that this stimulation may be mediated by increased responsiveness of the granulocyte-macrophage progenitors to the action of CSF. (Author abstract) (33 Refs)

45. DIFFERENTIATION OF FRIEND ERYTHROLEUKEMIC CELLS IN MEDIA LACKING FETAL CALF SERUM.

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Inst. Molecular Biology, Bulgarian Acad. Sciences, 1113 Sofia, Bulgaria
Neoplasma; 28(6):669-673 1981

The results presented in this paper provide evidence that the erythroid differentiation induced with n-butyric acid (BA) in F4N Friend cells depends mainly on the ability of differe nt media to support an active cellular proliferation. No significant differences in the percentage of hemoglobin-positive cells were observed when a comparative induction was performed in media, supplemented with either calf or fetal calf serum; the most satisfactory results were obtained in Dulbecco's minimum essential medium (DMEM), lacking fetal calf serum but containing calf serum instead. The great sensitivity of cells to BA in the presence of calf serum allows us to suggest that there is not any component, specific for the fetal calf serum, critically required for the induction of erythroid differentiation in this clone. (Author abstract) (15 Refs)

EXOGENOUS AGENTS AFFECTING DIFFERENTIATION

46. DNA LIGASE AND DNASE ACTIVITIES IN MOUSE ERYTHROLEUKEMIA CELLS DURING DIMETHYL SULFOXIDE-INDUCED DIFFERENTIATION.

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Cancer Res; 42(4):1300-1306 1982

DNA ligase and DNase levels were measured in cell-free extracts from untreated mouse erythroleukemia (MEL) cells and from cells treated with dimethyl sulfoxide (Me2SO) to induce erythroid differentiation. The DNase activity present in the extracts was sensitive to inhibition by G-actin and was, therefore, presumed to be DNase I. When the MEL cells were induced to differentiate by culturing in the presence of 1.8% Me2SO for 3 or 4 days, the apparent activity of DNA ligase decreased to approx 12% of the value in untreated MEL cells. In contrast, the apparent DNase I activity of the extracts from

Me2SO-treated cells increased over that in extracts from untreated cells by a factor of 2. The activity of acid phosphatase, a lysosomal enzyme, remained unchanged. When strain DR-10, a mutant of the MEL cells which does not undergo Me2SO-induced differentiation, was treated with Me2SO, the DNA ligase and DNase activities of extracts from these cells remained unchanged as compared to extracts from untreated DR-10 cells. Therefore, the marked decrease in the level of DNA ligase activity appeared to be related to the process of differentiation in the Me2SO-treated MEL cells. (Author abstract) (39 Refs)

47. STIMULATION OF DNA SYNTHESIS IN MURINE FIBROBLASTS BY THE TUMOUR PROMOTER TELEOCIDIN: RELATIONSHIP TO PHORBOL ESTERS AND VASOPRESSIN.

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Biochem Biophys Res Commun; 104(4):1159-1166 1982

The influence of the tumor promoter teleocidin (TL) on initiation of DNA synthesis was studied in quiescent Swiss 3T3 and other cells. As determined with the use of (3H)thymidine, TL at 20 nanoM (nM) markedly enhanced DNA synthesis at suboptimal concentrations of serum in Swiss 3T3 cells. In the p resence of 2.5% serum, which does not support growth of the 3T3 cells, TL (100 nM) stimulates confluent cells to form multiple layers after 4 days incubation. In serum-free medium, 20 nM TL stimulated DNA synthesis when added in combination with retinoic acid, epidermal growth factor, cholera toxin, platelet-derived growth factor, insulin, fibroblast-derived growth factor (FDGF), and phorbol-12,13-dibutyrate (PDBu). Synergism was shown with FDGF when both TL and retinoic acid were present. No synergism between PDBu and TL was shown in any of the cell types used. Several lines of evidence indicate that TL and phorbol esters bind to the same receptor, or their actions could converge at a post-receptor locus. Thus, prolonged treatment of Swiss 3T3 cells with PDBu leads to a marked decrease in (3H)-PDBu binding sites and marked reduction in subsequent mitogenic response not only to phorbol esters, but also to TL. TL is not synergistic with vasopressin, suggesting that it utilizes the same mitogenic pathway as this physiological ligand. (17 Refs)

48. MODIFICATION BY SERUM OF DIFFERENTIATION OF CULTURED HUMAN MYELOID LEUKEMIA CELLS IN RESPONSE TO 12-O-TETRADECANOYLPHORBOL-13-ACETATE.

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Gan; 73(1):97-104 1982

In medium with serum, 12-O-tetradecanoylphorbol-13-acetate (TPA) induced alpha-naphthyl acetate esterase activity in human promyelocytic leukemia cells (HL-60), adherence of the cells to the culture dish, and their change into forms that were morphologically similar to macrophages. HL-60 cells grew in the absence of serum in synthetic medium supplemented with insulin, transferrin, and several trace elements, and could be maintained for more than 6 mo in this medium. Induction of differentiation by TPA was observed with cells grown in serum-free medium. Human myeloid leukemia cells (K562-4) cultured in medium with serum could not be induced to differentiate even in the presence of TPA, but their differentiation into macrophages in the presence of TPA, arginase or actinomycin D was observed after they had been grown in serum-free medium for 4 mo. Addition of serum inhibited the induction of differentiation of HL-60 and K562-4 cells that had been grown in serum-free medium. Calf serum was more inhibitory than fetal calf serum on TPA-induced differentiation, but there was no significant difference in the effects of the two sera on induction by actinomycin D or arginase. These results suggest that the different responses in media with different sera may be specific to TPA. Induction of adhesiveness of K562-4 cells by TPA required some unknown serum factor(s), although addition of serum inhibited the inductions of morphological and functional differentiation. The relation between the ability of K-562-4 cells to be induced to differentiate into macrophages and long-term cultivation in serum-free medium is discussed. (Author abstract) (18 Refs)

49. PHORBOL ESTER-INDUCED DIFFERENTIATION OF A NON-T, NON-B LEUKEMIC CELL LINE: MODEL FOR HUMAN LYMPHOID PROGENITOR CELL DEVELOPMENT.

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J Immunol; 128(3):1316-1320 1982

The phorbol ester 12-O-tetradecanoylphorbol-13-acetate (TPA) was tested for its ability to induce phenotypic changes in the human non-T, non-B ALL cell line REH. Cells were cultured with nanogram concentrations of TPA for up to 48 hr, and were analyzed by indirect immunofluorescence with a panel of monoclonal antibodies and an antibody to the enzyme terminal deoxynucleotidyl transferase (TdT). TPA induced REH cells to express the leukemia-associated antigen, p24 (detected with monoclonal antibody BA-2; p24/BA-2) by 8 hr of culture, with induction complete by 24 hr. TPA-treated cells also underwent a concomitant decrease in the expression of TdT when analyzed enzymatically or by immunofluorescence. Analysis of TPA-treated cells with monoclonal antibodies BA-1 (detecting a B cell-associated antigen), 7.2 (detecting a monomorphic HLA-DR antigen), or OKT11 (detecting a structure closely associated with the E receptor) showed no change compared to controls. In addition there was no detectable cytoplasmic immunoglobulin in control or TPA-treated cells. These results show clearly that TPA is capable of inducing phenotypic changes in REH cells. Such changes may reflect the differentiation-linked expression of antigens present in normal bone marrow lymphoid progenitor cells. (Author abstract) (41 Refs)

50. EFFECTS OF PHORBOL ESTERS ON BASAL EPIDERMAL CELLS DERIVED FROM EAR SKIN OF ADULT GUINEA PIGS.

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Cancer Res; 42(5):1975-1979 1982

In cultures of basal epidermal cells obtained from ear skin of adult guinea pigs, the tumor-promoting phorbol ester 12-O-tetradecanoylphorbol-13-acetate induces cellular proliferation which is prostaglandin dependent and inhibited by indomethacin. Inhibition by indomethacin can be overcome by prostaglandins E2 and F2alpha, with prostaglandin F2alpha being more effective. The concomitant induction of ornithine decarboxylase activity is insensitive to indomethacin inhibition. The nonpromoting derivative 4-O-methyl-12-O-

tetradecanoylphorbol-13-acetate evokes a prostaglandin-independent, ie, indomethacin-insensitive, stimulation of cell proliferation without inducing ornithine decarboxylase activity. (Author abstract) (28 Refs)

51. DIFFERENT EFFECTS OF DIMETHYLBENZ(A)ANTHRACENE AND TETRADECANOYLPHORBOL ACETATE ON DIFFERENTIATION OF MAST CELLS IN THE SKIN OF MICE.

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Am J Pathol; 106(3):312-317 1982

The effects of the application of 7,12-dimethylbenz(a)anthracene (DMBA) and 12-O-tetradecanoylphorbol-13-acetate (TPA) on the differentiation of tissue mast cells were investigated. Giant granules of beige (C57BL/6-bgJ/bgJ, Chediak-Higashi syndrome) mice were used to distinguish different populations of mast cells. In the normal C57BL/6 mice that had been irradiated and injected with bone marrow cells of beige C57BL/6 mice (bgJ/bgJ to +/+ chimeras), mast cells in the skin remained normal in type and mast-cell precursors in the bone marrow and peripheral blood were of the beige type. When DMBA was painted onto the skin of such bgJ/bgJ to +/+ chimeras, the number of beige mast cells increased, but the number of normal mast cells did not change. On the other hand, the number of both normal and beige mast cells increased when TPA was applied to the bgJ/bgJ to +/+ chimeras. Autoradiographic study showed that beige mast cells proliferated more than normal mast cells after DMBA applications, whereas the proliferation was comparable in both types of mast cells after TPA applications. The present results indicate that the effects of DMBA applications on differentiation of mast cells are different from those of TPA applications. (Author abstract) (18 Refs)

52. IDENTIFICATION OF PHORBOL ESTER RECEPTORS IN T-CELL GROWTH FACTOR-PRODUCING AND - NONPRODUCING EL4 MOUSE THYMOMA CELLS.

Sando JJ, Hilfiker ML, PiacentiniMJ, Laufer TM
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Cancer Res; 42(5):1676-1680 1982

Two lines of EL4 mouse thymoma cells have been examined for the presence of specific phorbol ester-binding components. Both lines displayed saturable specific binding as determined by using [20-3H]phorbol 12,13-dibutyrate in a whole-cell-binding assay. Specific binding in each line was max within 10 min at 37 C but rapidly decreased to about 30% within 30 min. Max binding at 4 C was reached after 60 min and was stable for at least 8 hr; however, this level was only about 30% of the max obtained at 37 C. Saturation of the specific binding after 3 hr at 4 C occurred at a concentration (30 to 50 nM) which is consistent with that yielding max T-cell growth factor production in the responding line but greater than that at which inhibition of proliferation was detected in those cells. Scatchard analysis of these data was consistent with the existence of a single class of binding sites with a Kd of 11 +-0.6 (SD) nM for the T-cell growth factor-producing cells and 18 +-1.9 nM for the nonproducing line. The numbers of sites per cell measured in the responding and nonresponding lines were 5.6 +-1.3 x 10(4) and 7.1 +-0.2 x 10(4), respectively. Competition by a series of phorbol esters for [20-73 1H]phorbol 12,13-dibutyrate binding in the responding line showed the same order of potency as production of T-cell growth factor and inhibition of proliferation by the analogs. These data support identification of the phorbol ester-binding component in the responding cells as the receptor mediating T-cell growth factor production. The presence of binding components in unresponsive cells in numbers and with characteristics indistinguishable from those of responsive cells suggests that the failure to respond is due to modification of a step which succeeds the initial binding event. (29 Refs)

53. CA-CALMODULIN MEDIATES THE DNA-SYNTHETIC RESPONSE OF CALCIUM-DEPRIVED LIVER CELLS TO THE TUMOR PROMOTER TPA.

Jones A, Boynton AL, Macmanus JP, Whitfield JF
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Exp Cell Res; 138(1):87-93 1982

Incubation in low (0.02 mM)-calcium medium inhibited the proliferation of T51B rat liver cells. Addition of TPA (12-O-tetradecanoyl-phorbol-13-acetate; 50 ng/ml) or calcium (1.25 mM) caused the arrested, calcium-deprived cells to initiate DNA synthesis within 2 hr and eventually to resume proliferating. The rapid DNA-synthetic response to TPA addition appeared to depend on the small amount of calcium remaining in the calcium-deficient medium, because it was blocked by correspondingly small amounts of the specific calcium chelator EGTA or La+++, which displaces calcium from the cell surface and inhibits transmembrane calcium fluxes. The common mediation of TPA and calcium actions through Ca-calmodulin complexes was indicated by the following observations: The DNA-synthetic responses to both agents were blocked by the supposedly specific Ca-calmodulin blocker trifluoperazine; the trifluoperazine-induced block was overcome by pure calmodulin; and the responses to both TPA and calcium were blocked by anti-calmodulin antibodies. (Author abstract) (35 Refs)

54. THE MECHANISM OF ACTION OF CARCINOGENS AND TUMOR PROMOTERS AND THEIR RELEVANCE TO CANCER PREVENTION.

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Div. Environmental Sciences, Columbia Univ. Coll. Physicians and Surgeons, New York, NY, 10032
Cancer Research in the People's Republic of China and the United States of America: Epidemiology, Causation and New Approaches to Therapy, Proceedings of a Conference held in New York, 28-29, March, 1980. Marks PA, ed. New York, Grune and Stratton, 273 pp., 1981.

Systems for studying malignant cell transformation in vitro, mimicry of transformation, modulation of differentiation, membrane effects and phospholipid metabolism, membrane receptor s for epidermal growth factor and 12-O-tetradecanoyl-phorbol-13-acetate (TPA), synergistic interactions between chemical agents and viruses in cell transformation, molecular mechanism of initiation, and a unified theory of initiation and promotion are discussed. Examples of cell culture systems that can be transformed by chemical carcinogens and by TPA modulation of differentiation are presented. (61 Refs)

55. CONVERSION OF MALIGNANT MURINE EMBRYONAL CARCINOMAS TO BENIGN TERATOMAS BY CHEMICAL INDUCTION OF DIFFERENTIATION IN VIVO.

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Cancer Res; 42(5):1843-1849 1982

PCC4azal embryonal carcinoma tumors were grown in strain 129 mice by sc transplantation. When palpable, the tumors were treated with a combination of retinoic acid and dimethylacetamide. In vitro, this embryonal carcinoma cell line shows minimal spontaneous differentiation and is exquisitely sensitive to retinoic acid and/or dimethylacetamide induction of differentiation. Ten daily 20-ul intratumor injections of a solution of 10 mg retinoic acid per ml of dimethylacetamide resulted in nearly complete induction of morphological differentiation mainly into neuroepithelial and glandular derivatives. Control tumors showed minor spontaneous differentiation. Differentiation was associated with decreased tumor growth rate, decreased mitotic index, decreased extent of necrosis, and increased survival time of the hosts. In 4 of 18 cases, long-term survival of the hosts was effected by a complete differentiation of the malignant embryonal carcinoma tumors into benign teratomas. Retinoic acid:dimethylacetamide was also effective in inducing differentiation with the same dosage and schedule when administered systemically, ie, ip or sc. (Author abstract) (23 Refs)

56. EFFECT OF BENZENE METABOLITES ON PHYTOHEMAGGLUTININ-STIMULATED LYMPHOPOIESIS IN RAT BONE MARROW.

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J Reticuloendothel Soc; 31(2):155-170 1982

Chronic exposure of animals to benzene results in lymphocytopenia and bone marrow depression. A highly sensitive microculture system was developed to amplify and characterize marrow mitogen response which depended on the isolation of cells by Percoll continuous density gradient centrifugation and strict adherence to optimal culture conditions. Maximal mitogen responses to phytohemagglutinin (PHA) and concanavalin A (con A), as assessed by (3H)-thymidine uptake, occurred at later times in rat bone marrow cultures (Days 5-7 of culture) compared with spleen and thymus (Days 2-3). Compared with spleen and thymus, the PHA:con A stimulation ratio was inverted for marrow, yet the responsive cells were morphologically identical to those of peripheral lymphoid organs. Populations enriched in lymphoid precursors were inhibited from responding to PHA stimulation at noncytotoxic concentrations (greater than uM) of the benzene metabolites, p-benzoquinone or 1,2,4-benzenetriol. Pretreatment with less than uM concentrations of metabolites resulted in a modulation of lectin-induced responses such that blastogenesis was induced at higher concentrations of lectin relative to untreated cells. These changes indicate that known metabolites of benzene induce a concentration dependent modulation of differentiation and proliferation in lectin-stimulated cells from rat bone marrow in vitro. (Author abstract) (56 Refs)

57. NAFAZATROM (BAY G 6575) INHIBITION OF TUMOR CELL LIPOXYGENASE ACTIVITY AND CELLULAR PROLIFERATION.

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FEBS Lett; 139(1):65-68 1982

The effects of nafazatrom (Bay g 6575; 1-[(2-beta-naphtyloxy)ethyl]-3-methyl-2-pyrazolin-5 one) on proliferation of B16 amelanotic melanoma cells in culture were examined over a period of 14 days. Media was changed and drug was added every other day. A dose-dependent decrease in cell proliferation was observed with a minimal effective dose of 0.1 ug/ml. This effect of nafazatrom was not due to cytotoxicity. Nafazatrom also decreased [3H]thymidine incorporation into DNA. Effects of nafazatrom were examined in vivo and on tumor cell arachidonic acid metabolism. The drug inhibited lipoxygenase activity but not prostaglandin endoperoxide synthetase (cyclooxygenase + hydroperoxidase). (16 Refs)

58. EFFECTS OF HEXAMETHYLENE BISACETAMIDE ON ALPHA-FETOPROTEIN, ALBUMIN, AND TRANSFERRIN PRODUCTION BY TWO RAT HEPATOMA CELL LINES.

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In Vitro; 18(2):157-164 1982

Alpha-Fetoprotein (AFP), albumin, and transferrin production by two rat hepatoma cell lines, McA-RH 7777 (7777) and McA-RH 8994 (8994), was determined after treatment with hexamethylene bisacetamide (HMBA). Radioimmunoassays were used to determine the levels of both secreted and intracellular AFP, albumin, and transferrin. After treatment with HMBA for a period of 28 to 96 h a threefold increase in AFP secretion by 7777 and a dose related increase in AFP, albumin, and transferrin secretion by 8994 were observed. In contrast, the secretion of albumin and transferrin in 7777 was inhibited by 60 and 40%, respectively, following treatment with HMBA. The intracellular concentrations of AFP in 7777 and AFP, albumin, and transferrin in 8994 were increased by treatment with HMBA indicating that HMBA is able to stimulate the synthesis of these proteins. The intracellular concentration of AFP, albumin, an d transferrin in 7777, when expressed as a percentage of the extracellular concentration of these proteins, did not change significantly during HMBA treatment, indicating that the observed decrease in secreted albumin and transferrin by 7777 is due to decreased synthesis. Similarly, in Line 8994, when the intracellular concentration of the three proteins was expressed as percentage of the extracellular concentration, the only significant change observed was an increase in AFP after 72 hr of HMBA (5 mM) treatment. The observed changes in the synthesis of AFP, albumin, and transferrin in both 7777 and 8994 after HMBA treatment were reversible, as judged by the return to control values upon removal of HMBA from the culture medium. Thus, HMBA stimulates synthesis of the oncofetal protein AFP, a result that appears to be independent of the stage of differentiation of the cell. However, its effect on the synthesis of albumin and transferrin are opposite in the two cell lines, suggesting that the regulation of the synthesis of these two proteins is controlled by factors or conditions that are dependent upon the stage of differentiation of the hepatoma cell lines. (33 Refs)

59. MODULATION OF ASCITES TUMOUR GROWTH BY NUCLEIC ACIDS AND NUCLEASES.

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Br J Cancer; 45(2):295-299 1982

Effects of DNA and RNA and DNase and RNase on ascites tumor growth in vitro and in vivo were investigated. The considerable cell loss occurring during development of the Krebs 2 tumor may cause the high levels of DNA and RNA fragments in ascitic fluid. Addition of 300 ug/ml DNA (calf thymus) beneficially affected growth of tumor cells from 2-day-old tumors; prior treatment of the added DNA with DNase I abolished stimulation and heat denaturation of the DNA reduced stimulation. The ability of cells from 2-day-old tumors to grow in culture following pretreatment with high levels of electrophoretically purified DNase I sufficient to digest DNA of most of the nucleoprotein complexes in the extracellular ascites fluid was studied. Such treatment depressed the growth of tumor cells. Pretreatment with high levels of RNase A also depressed growth of tumor cells. Addition of 1 mg/ml DNase I, 2 mg/ml RNase, or 300 ug/ml DNA had no significant effect on growth of 3-day cultured L-929 mouse fibroblasts. Extent of tumor growth in vivo was measured by relative wt gain in mice inoculated with Krebs 2 tumor. Tumor growth was enhanced by the addition of 100 ug portions of DNA ip; RNA inoculation had a varied and less pronounced stimulatory effect. Pretreatment of tumor inoculum with DNase or RNase resulted in subsequent body wt gain, but only half the normal. Tumor development was retarded by successive administration of RNase or DNase after a delay of 24 hr after inoc ulation of tumor cells; a delay of 7 days reduced tumor growth to half. RNase was always slightly more effective than DNase in the in vivo tests, perhaps because DNase is labile in ascites fluid at 37 C. (14 Refs)

60. EFFECT OF MAGNESIUM CONTENT ON DENSITY-DEPENDENT REGULATION OF THE ONSET OF DNA SYNTHESIS IN TRANSFORMED 3T3 CELLS.

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Cancer Res; 42(5):1761-1768 1982

A spontaneously transformed clone of BALB/c 3T3 cells became more transformed after more than 90 passages as indicated by increased rounding of cells, multiplication to a higher saturation density, and increased ability to form colonies when suspended in agar. When the extracellular concentration of Mg++ was sharply reduced, the highly transformed cells flattened, assumed the shape of nontransformed cells, and became regularly arranged in cohesive arrays. If crowded when deprived of Mg++, they lost more intracellular Mg++ than did nontransformed and early passage-transformed cells and remained at constant cell density for at least 10 days. The intracellular content of neither Na+ nor K+ changed consistently, with Mg++ deprivation, but the Ca++ content increased more than two-fold. The sensitivity of the onset of DNA synthesis to inhibition by Mg++ deprivation increased with the extent of crowding of the cultures. This was demonstrated by varying population density within a single culture dish as well as from culture to culture. The loss of intracellular Mg++ in low concentrations of extracellular Mg++ increased with cell crowding as did the inhibition of DNA synthesis per fractional loss of intracellular Mg++. Neither deprivation of K+ or Ca++ nor addition of cyclic adenosine 3':5'-monophosphate produced a density-dependent inhibition of DNA synthesis. The results indicate that a reduction of the Mg++ content of highly transformed cells restores density-dependent inhibition of the onset of DNA synthesis, which is a characteristic property of nontransformed cells. The differences in Mg++ retentiveness with population density may reflect differences of intracellular distribution and binding of Mg+, which could in turn explain some of the regulatory effects of population density on metabolism and growth. (Author abstract) (16 Refs)

61. INHIBITION OF CELLULAR DIVISION OF A MURINE MACROPHAGE TUMOR BY MACROPHAGE-ACTIVATING AGENTS.

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Cancer Metastasis and Treatment Lab., NCI-Frederick Cancer Res. Center, Frederick, MD, 21701
Cancer Res; 42(5):1850-1855 1982

Although the murine reticulum cell sarcoma M5076 is highly malignant in vivo, in vitro it displays many of the functional characteristics of an activated macrophage, such as phagocytosis and tumor cytotoxicity. This study was designed to determine what effect macrophage-activating agents would h ave on the function and growth of M5076 cells. Exposure of M5076 tumor cells to substances that activate normal macrophages to the tumoricidal state rapidly and irreversibly induced cessation of cellular division. The treated tumor cells, however, retained the same characteristics as those of untreated M5076 cells in vitro with respect to viability and the activated macrophage functions of phagocytosis and tumor cytotoxicity. Even after a short exposure to lipopolysaccharide, the ability of M5076 cells, injected iv into syngeneic mice, to form tumor nodules was greatly reduced. These results indicate that a highly malignant tumor of macrophage origin, M5076, can be induced to cease cellular division while retaining the functional attributes of an activated macrophage. We speculate that the exposure of M5076 to macrophage-activating agents results in the induction of terminal differentiation of this tumor. (Author abstract) (30 Refs)

62. EFFECTS OF HYPERTHERMIA ON DIVIDING CHINESE HAMSTER OVARY CELLS AND ON MICROTUBULES IN VITRO.

Coss RA, Dewey WC, Bamburg JR
Dept. Radiation Therapy and Nuclear Medicine, Thomas Jefferson Univ. Hosp., Philadelphia, PA, 19107
Cancer Res; 42(3):1059-1071 1982

The ultrastructure of Chinese hamster ovary cells was examined immediately after heating cells in mitosis, and the findings were compared with (a) the behavior of heated cells monitored with time lapse cinematography following heat shocks and (b) proliferative survival of individual cells followed for 7 days after heating. Treatment of dividing cells at 45.5 C (5 to 15 min) disassembled the spindle and disrupted both the contractile ring and the midbody-cytoplasmic bridge complex to varying degrees depending on the length of heating. The spindle did not reform upon return to 37.0 C. Microtubular proteins were heated in vitro to determine if their inactivation was responsible for the inability of the spindle to reform. Heat completely disassembled the intact microtubules and inactivated a proportion of the microtubular proteins in vitro; however, a fraction of the microtubular proteins from heat-disassembled microtubules still was capable of reassembly. The time lapse studies indicated that cells in division at the time of heating entered G1 without completing division. Of the resultant tetraploid cells, 88% had greater than or equal to 2 nuclei; 59% of the tetraploid cells divided 35 +-7 (S D) hr following the heat

shock (control generation time, 13 +-2 hr), and 95% of the flattened progeny had more than one nucleus. The fate of individual cells that were in mitosis or G1 when treated at 45.5 C for 4.5 min was monitored for 7 days. The survival of the total heated population of cells was 19%, but the surviving cells were almost totally accounted for by the G1 cells present as contaminants in the heated population. Less than 2% (2 of 115) of the monitored cells that were heated in mitosis formed colonies. Therefore, the morphological disruption of the spindle, contractile ring, or midbody-cytoplasmic bridge complex by a heat of 45.5 C prevented cytokinesis, and the resultant tetraploid cells became proliferatively dead. (Author abstract) (34 Refs)

OTHER RELATED STUDIES

63. INHIBITION OF GROWTH OF HUMAN CANCERS BY EXTRACTS FROM TRICHOPHYTON VERRUCOSUM.

Floersheim GL, Looser R, Grundmann HP, Meyer JC
Lab. Transplantation Biology, Dept. Surgery, Kantonsspital, Hebelstrasse 20, 4031 Basle, Switzerland
Lancet; 1(8274):708-710 1982

Lyophilized extracts from cultured dermatophytes were injected into mice carrying rapidly proliferating xenografts of a human Ewing sarcoma or a human colon carcinoma. Extracts from strains of Trichophyton verrucosum greatly inhibited the growth of both tumors. There were no overt signs of toxicity. (Author abstract) (15 Refs)

64. CURRENT CANCER RESEARCH ON CELL DIFFERENTIATION, TUMOR GROWTH, INVASION, AND METASTASIS (SPECIAL LISTING).

International Cancer Research Data Bank Program Office
NCI, Westwood Building, Room 10A18, Bethesda, MD, 20205
Cell Differentiation, Tumor Growth, Invasion, and Metastasis (Special Listing) This Title is Available Through National Technical Information Service, Springfield, VA, as NTISUB/PB81-929903:; 76pp. 1981

Special Listings of Current Cancer Research Projects are a service of the International Cancer Research Data Bank (ICRDB) program of the National Cancer Institute. Each listing contains descriptions of ongoing projects in one selected cancer research area. The descriptions are provided by cancer scientists in about 50 different countries. Régulation of tumor cell differentiation and growth is discussed with respect to genetic and epigenetic mechanisms, cell surface components and membrane function, hormones and other factors, cyclic nucleotides, and polyamine regulation of growth. Differentiation and growth in normal and leukemic hematopoietic cell differentiation and growth (differentiation of hematopoietic stem cells, RBC, and lymphocytes) and in embryonal carcinoma cells is reviewed. Cell differentiation and proliferation is described. Mechanisms and characteristics of tumor vascularization and invasion and metastases are outlined, including the role of enzymes in invasion and metastasis (clotting and fibrinolytic enzymes, collagenolytic enzymes, and other proteolytic enzymes and destructive substances in tumor invasion and growth), cell surface components and cell-cell interactions, immunological mechanisms involved in metastases, chemotaxis and locomotion of tumor cells, and other studies.

65. GROWTH OF HYBRIDOMA CELLS IN SERUM-FREE MEDIUM: ETHANOLAMINE IS AN ESSENTIAL COMPONENT.

Murakami H, Masui H, Sato GH, Sueoka N, Chow TP, Kano-Sueoka T
Dept. Food Sciences, Univ. Kyushu, Fukuoka, Japan
Proc Natl Acad Sci USA; 79(4,part1):1158-1162 1982

A serum-free medium supplemented with a few growth factors was devised to grow lymphocyte hybridomas. The medium was developed with the hybridoma line MPC11-BL, a fusion product between a mouse plasmacytoma cell line (MPC11TG70na3) and mouse (BALB/c) spleen cells. In the process of developing the medium, ethanolamine was found to be an essential growth factor for the hybridoma. Phosphoethanolamine at 10-fold higher concentration could substitute for ethanolamine. Long-term cultivation of the cells was achieved in the defined medium supplemented with insulin, transferrin, ethanolamine, and selenium. The defined medium supported the growth of various other mouse hybridoma cell lines, mostly at a rate comparable to that observed in a serum-containing medium. After one-step ammonium sulfate precipitation of the spent medium, more than 95% of the protein recovered was immunoglobulin as shown by NaDodSO4/polyacrylamide gel electrophoresis. (Author abstract) (16 Refs)

66. STIMULATION OF SIALIDASE ACTIVITY DURING CELL DIFFERENTIATION OF HUMAN PROMYELOCYTIC LEUKEMIA CELL LINE HL-60.

Nojiri H, Takaku F, Tetsuka T, Saito M
(c/o Saito) Dept. Hematology, Jichi Medical Sch., Yakushiji, Minami-Kawachi-machi, Kawachi-gun, Tochigi 329-04, Japan
Biochem Biophys Res Commun; 104(4):1239-1246 1982

Sialidase activity (SA) was assayed in sonicated cells of the human promyelocytic leukemia line HL-60 by a modification of the fluorometric method which used 2'-(4-methylumbelliferyl)alpha-D-N-acetylneuraminic acid as a substrate. SA activity of the cells had an acidic optimum at pH 4.1, with an indication of a second peak at pH 5.2. The SA activity was linear with up to 500 ug protein of cell homogenate and with time up to 120 min. The apparent Km value was 0.10 milliM. When the HL-60 cells were induced to differentiate into granulocytes with either 1 uM retinoic acid (RA) or 1.3% dimethylsulfoxide (DMSO), growth was markedly suppressed. After incubation of HL-60 cells for 6 days with RA and 8 days with DMSO, 91% and 75% of total cells, respectively, differentiated into morphologically mature myeloid cells and SA increased to 2.5-2.7x as much as that of controls. In human myeloid leukemia cell lines, K562 and KG-1, which also show some potential for cell differentiation, SA was found to be 1.5- and 3.8-fold that of HL-60, respectively. (20 Refs)

67. DIFFUSION CHAMBER (DC) CULTURING OF HAEMOPOIETIC CELLS: A RELIABLE ASSAY SYSTEM FOR REGULATORS OF PROLIFERATION?

Benestad HB, Toogood EO
Inst. Physiology, Karl Johansgt. 47, Oslo 1, Norway
Exp Hematol; 10(2):161-171 1982

If ip DC culturing of hemopoietic cells shall serve as a reliable assay for systemic and/or local peritoneal regulators of proliferation, cell growth must not be restricted by the diffusion capacity through the chamber membranes. Diffusion was assessed by measuring uptake in DC or 51Cr-EDTA of 3H-thymidine, given intravenously to mouse hosts or to chambers

incubated in vitro. Fractional incorporation of 3H-thymidine, numbers of mouse spleen colony-forming stem cells, agar colony-forming progenitors (of granulocytes and macrophages), and total cell numbers were taken to reflect cell growth. Changing the diffusion capacity did not not lead to marked changes in cell growth; and vice versa, culturing cells under host or chamber conditions that increased cell growth was not associated with an appreciably increased diffusion capacity. Diffusion was increased by replacing Acropor with Millipore DC or by repeatedly cleaning the DC during the culture period. Cell growth was increased by using irradiated rather than normal host mice, and Nuclepore rather than Millipore DC in irradiated mice. Increasing the oxygen delivery to the cultured cells by using polycythemic and hyperoxic hosts did not enhance cell growth either. (Author abstract) (20 Refs)

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SERIES CB16 No. 82/09 SEPTEMBER, 1982

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CELL AND SERUM DERIVED GROWTH EFFECTORS

1. EPIDERMAL GROWTH FACTOR INHIBITS GROWTH OF A431 HUMAN EPIDERMOID CARCINOMA IN SERUM-FREE CELL CULTURE.

Barnes DW
Dept. Biological Sciences, Univ. Pittsburgh, Pittsburgh, PA, 15260
J Cell Biol; 93(1):1-4 1982

A medium consisting of a rich basal nutrient mixture supplemented with bovine insulin (10 ug/ml), human transferrin (10 ug/ml), human cold-insoluble globulin (5 ug/ml), and ethanolamine (0.5 mM) supported the growth of the A431 human epidermoid cell line in the absence of serum with a generation time equal to that of cells in serum-containing medium. Addi-

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tion of epidermal growth factor (EGF) to this culture medium at concentrations mitogenic for other cell types resulted in a marked inhibition of A431 cell growth. Inhibitory effects of EGF were observed at 1 ng/ml and near-maximal effects were observed at 10 ng/ml. The inhibitory effect of EGF could be reversed by the omission of EGF in subsequent medium changes and could be prevented by the addition of anti-EGF antibody to the culture medium. Inhibition of A431 cell growth by EGF also could be demonstrated in serum-containing medium. (Author abstract) (34 Refs)

2. RESISTANCE TO PHOSPHATASE OF THIOPHOSPHORYLATED EPIDERMAL GROWTH FACTOR RECEPTOR IN A431 MEMBRANES.

Cassel D, Glaser L
Dept. Biological Chemistry, Div. Biology and Biomedical Sciences, Washington Univ. Sch. Medicine, St. Louis, MO, 63110
Proc Natl Acad Sci USA; 79(7):2231-2235 1982

Epidermal growth factor (EGF) increases the phosphorylation of its receptor and other membrane proteins, and these proteins can be rapidly dephosphorylated by membrane-bound protein phosphatase. We report that [35S]adenosine 5'-[gamma-thio]triphosphate is as effective as [gamma-32P]ATP as substrate for the EGF receptor-associated protein kinase in A431 membranes. Both the kinetics and the extent of the EGF-dependent thiophosphorylation at 0 C are similar to those obtained with [gamma-32P]ATP, provided that ATP hydrolysis by the membrane preparation is inhibited by addition of adenosine 5'-[beta,gamma-imino]triphosphate. The thiophosphorylation reaction requires Mn++ but differs from the phosphorylation reaction in the inability of Mg++ to serve as cofactor. Both EGF-dependent phosphorylated and thiophosphorylated membrane proteins yield the same two major bands of Mr 145,000-160,000 in autoradiograms of Na-DodSO4/polyacrylamide gel electrophorograms. The rate of dephosphorylation of membrane proteins that have been thiophosphorylated in the presence of EGF is dramatically slower (factors of 1/20 to 1/40) than that of the phosphorylted proteins at both 0 C and 32 C. This increased metabolic stability of the thiophosphorylated proteins will be useful for investigation of the role of phosphorylation in the biological effects of EGF. (Author abstract) (22 Refs)

3. INHIBITION OF EPIDERMAL GROWTH FACTOR BINDING TO MOUSE CULTURED CELLS BY FIBROBLAST-DERIVED GROWTH FACTOR. EVIDENCE FOR AN INDIRECT MECHANISM.

Rozengurt E, Collins M, Brown KD, Pettican P
Imperial Cancer Res. Fund, Lincoln's Inn Fields, London WC2A 3PX, England
J Biol Chem; 257(7):3680-3686 1982

Fibroblast-derived growth factor (FDGF), a basic, heat- and acid-stable polypeptide partially purified from the serum-free conditioned medium of BHK cells transformed by simian virus 40, is a potent mitogen for Swiss 3T3 cells and causes a marked reduction in 125I-labeled epidermal growth factor (125I-EGF) binding to these cells. The activity which inhibits EGF binding co-elutes with the growth-stimulating activity after gel filtration, ion exchange chromatography, and sodium dodecyl sulfate polyacrylamide gel electrophoresis. Both cellular responses are elicited by the same range of FDGF concentration in several murine cell types. The inhibition of EGF binding is rapid and results from a decrease in the apparent affinity of cellular receptors for 125I-EGF. FDGF does not affect the rate of cell-mediated 125I-EGF degradation. Several lines of evidence suggest that FDGF does not bind directly to EGF receptors. First, the effect of FDGF is dependent on the temperature of the assay; furthermore, treatment of cells with EGF results in loss of EGF receptors while exposure to FDGF from up to 24 hr does not induce 'down-regulation' of EGF receptors. Further, in A431 cells which display a large number of specific EGF receptors, 125I-EGF binding is not sensitive to FDGF. Finally, the effect of FDGF on 125I-EGF binding is not observed with isolated plasma membranes. Taken together, these findings suggest that FDGF binds to sites which are separate from EGF receptors. The results show a novel mechanism whereby a growth-promoting factor produced by a tumor cell line can rapidly modulate the affinity of the cellular receptors from EGF in an indirect manner. (Author abstract) (46 Refs)

4. ALTERED EPIDERMAL GROWTH FACTOR (EGF)-STIMULATED PROTEIN KINASE ACTIVITY IN VARIANT A431 CELLS WITH ALTERED GROWTH RESPONSES TO EGF.

Buss JE, Kudlow JE, Lazar CS, Gill GN
(c/o Gill) Dept. Medicine, Div. Endocrinology, Univ. California San Diego Sch. Medicine, La Jolla, CA, 92093
Proc Natl Acad Sci USA; 79(8,part1):2574-2578 1982

To determine the role of epidermal growth factor (EGF)-stimulated protein kinase in the biological effects caused by EGF, tyrosine-specific kinase activity has been quantitated in A431 human epidermoid carcinoma cells and six variant cell lines. Because EGF inhibited proliferation of A431 cells, variants resistant to this inhibition were selected by treatment with mutagen and maintenance for 1 month in 0.1 uM EGF. After cloning and growth for 6-20 generations without EGF, the resistance of the variants to the growth-inhibitory effect of EGF was confirmed. Whereas EGF increased cellular phosphotyrosine content approx 10-fold in parental A431 cells, EGF caused smaller or undetectable increases in the six variant cell lines. Solubilized membranes from the six variants displayed diminished EGF-stimulated phosphorylation of the EGF receptor and of antibodies to p60src (the product of the Rous sarcoma virus transforming gene), which act as an exogenous substrate. The decrease in EGF-stimulated tyrosine-specific protein kinase activity varied from approx 40% (Clone 16) to approx 8% (Clone 18) of parental A431 activity. Phosphorylated EGF receptors from parental and variant cells migrated identically on sodium dodecyl sulfate/polyacrylamide gels. The number of EGF receptors in variant cells decreased in parallel with EGF-stimulated protein kinase activity, so that the specific activity of EGF-stimulated protein kinase per EGF receptor remained constant in the six variant cell lines with reductions in both activities to as low as 10%. These results suggest that this tyrosine-specific protein kinase activity mediates the growth-inhibitory effect of EGF on A431 cells and that both EGF binding and kinase activities reside in the same or tightly associated molecules. (Author abstract) (30 Refs)

5. NERVE GROWTH FACTOR: ACTIVATION OF THE CLASSICAL COMPLEMENT PATHWAY BY SPECIFIC SUBSTITUTION FOR COMPONENT C1.

Boyle MD, Young M
Dept. Immunology and Medical Microbiology, Univ. Florida Coll. Medicine, Gainesville, FL, 32610
Proc Natl Acad Sci USA; 79(8,part1):2519-2522 1982

The interaction of homogeneous preparations of mouse submandibular gland nerve growth factor (NGF) with the classi-

cal complement pathway was studied. NGF was found to be capable of carrying out the enzyme activities of the first component (C1) of the classical complement pathway (ie, the cleavage of zymogen C4 and C2). NGF would not substitute for any other classical pathway component, C2-C9. The C1-like activity of NGF was inhibited by human C1 inactivator. This interaction of NGF with the complement system may account for the previously described ability of NGF to accelerate the rate of contraction of experimentally induced wounds. (Author abstract) (25 Refs)

6. AN EFFECT OF FIBRONECTIN IN EPIDERMAL GROWTH FACTOR (EGF)-STIMULATED MEMBRANE PROTEIN PHOSPHORYLATION IN HUMAN CARCINOMA CELLS.

Rieber M, Rieber MS

Centre Microbiology and Cell Biology, Instituto Venezolano de Investigaciones Cientificas, Caracas 1010-A, Venezuela

Exp Cell Res; 138(2):441-446 1982

Membrane components of about 20 kd (kilodalton) which appear weakly phosphorylated in protein kinase assays of A431 human carcinoma plasma membrane to which epidermal growth factor (EGF) is added, increase their relative phosphorylation when human serum fibronectin is also included in the assay. Membranes prepared from 32PO4-labeled cells exposed to EGF and fibronectin prior to organelle isolation revealed the presence of an anodic 20 kd component not detected in cells which were not exposed to fibronectin. No comparable effects were apparent with bovine heart protein kinase and with membrane-associated protein kinase preparations which were not responsive to EGF. (Author abstract) (17 Refs)

7. FIBRONECTIN PROMOTES RAT SCHWANN CELL GROWTH AND MOTILITY.

Baron-van Evercooren A, Kleinman HK, Seppa HE, Rentier B, Dubois-Dalcq M

Infectious Diseases Branch, Natl. Inst. Neurological and Communicative Disorders and Stroke, NIH, Bethesda, MD, 20205

J Cell Biol; 93(1):211-216 1982

Techniques are now available for culturing well characterized and purified Schwann cells. Therefore, we investigated the role of fibronectin in the adhesion, growth, and migration of cultured rat Schwann cells. Double-immunolabeling shows that, in primary cultures of rat sciatic nerve, Schwann cells (90%) rarely express fibronectin, whereas fibroblasts (10%) exhibit a granular cytoplasmic and fibrillar surface-associated fibronectin. Secondary cultures of purified Schwann cells do not express fibronectin. Exogenous fibronectin has a small effect on promoting the adhesion of Schwann cells to the substrate and does not significantly affect cell morphology, but it produced a surface fibrillar network of fibronectin on the secondary Schwann cells. Tritiated thymidine autoradiography revealed that addition of fibronectin to the medium, even at low concentrations, markedly stimulates Schwann cell proliferation, in both primary and secondary cultures. In addition, when cell migration was measured in a Boyden chamber assay, fibronectin was found to moderately, but clearly, stimulate directed migration or chemotaxis. (Author abstract) (38 Refs)

8. ROLE OF FIBRONECTIN DEGRADATION PRODUCTS ON CELLULAR TRANSFORMATION.

Barlati S, De Petro G, Vartio T, Vaheri A

Istituto di Genetic Biochimica ed Evoluzionistica CNR, 27100 Pavia, Italy

Protides Biol Fluid Proc Colloq; 29:303-306 1982

The possible association of transformation enhancing factor (TEF) activity with fibronectin (FN) and/or fibrinogen (FG) degradation products [obtained by plasmin digestion of purified human plasma FN (FNdP) and FG] was studied. Plasminolytic fragments of FN but not of FG have potent TEF activity associated with the aggregated fragments of FN but not with the N-terminal of 30 kilodalton fragments carrying the transglutaminase substrate site. The potent TEF activity of FNdP is mimicked by 12-O-tetradecanoylphorbol-13-acetate, widely used as a prototypic tumor promoter. (10 Refs)

9. POSSIBLE ROLE OF THE PLASMA MEMBRANE IN REGULATING CELL GROWTH BAHVIOUR.

Ballmer K, Burger MM

Dept. Biochemistry, Biozentrum, Basel, Switzerland

Protides Biol Fluid Proc Colloq; 29:307-311 1982

After binding of a nontoxic lectin, succinylated concanavalin A (SCA), to the plasma membrane of normal and transformed cells in culture, both cell types responded to the growth inhibitory effect of SCA in a reversible, cell density-dependent manner. Inhibition occurred in the G0/G1 phase. When cells were grown in the presence of iodinated epidermal growth factor (EGF) without any other mitogens, reversible inhibition of EGF-binding as well as of EGF-stimulated DNA synthesis was observed. (11 Refs)

10. TRANSFERRIN RECEPTORS IN TRANSFORMATION AND DIFFERENTIATION.

Yeh CJ, Faulk WP

Blond McIndoe Centre for Transplantation Biology, Queen Victoria Hosp., East Grinstead, Sussex RH19 3DZ, England

Protides Biol Fluid Proc Colloq; 29:451-454 1981

Human promyelocytic leukemia HL-60 cells, Burkitt's lymphoma Daudi cells, and myelogenous leukemia K562 cells were grown with 1.25% dimethylsulfoxide (DMSO), 0.1% sodium n-butyrate (SnB), or 1 x 10(-6) retinoic acid (RA) for 4 days at 37 C, and transferrin receptors and growth were examined. HL-60 cells originally consisted of myeloblasts and promyelocytes and differentiated to metamyelocytes and banded neutrophils following induction; Daudi and K562 cells were unchanged. The indirect immunofluorescence test revealed that all three cell lines were strongly reactive for transferrin receptors, but HL-60 cells were weakly reactive after culture with DMSO, SnB, or RA; Daudi and K562 cells remained strongly reactive. Growth of HL-60 cells was strongly inhibited by all three agents, Daudi cells by SnB, and K562 cells by RA; K562 cells were barely affected by SnB. These findings indicate a role for transferrin receptors in differentiation and transformation: loss of receptor activity may be interpreted as being an index of change from immature to mature or from malignant to normal. (18 Refs)

11. STUDIES OF THE TRANSFERRIN RECEPTOR ON BOTH HUMAN RETICULOCYTES AND NUCLEATED HUMAN CELLS IN CULTURE. COMPARISON OF FACTORS REGULATING RECEPTOR DENSITY.

Frazier JL, Caskey JH, Yoffe M, Seligman PA
Div. Hematology/Oncology, Dept. Medicine, Univ. Colorado Sch. Medicine, Denver, CO, 80262
J Clin Invest; 69(4):853-865 1982

The transferrin receptor, present on reticulocytes and nucleated cells in tissue culture, has been measured with both immunoassay techniques and transferrin binding studies. The total cellular immunoreactive receptor is rapidly lost from erythrocytes during the process of reticulocyte maturation. This event parallels the loss of cell surface transferrin binding sites and RNA content, and correlates with previous studies that have measured the decline in hemoglobin synthesis. Nonhemoglobin-producing normal human fibroblasts, which appear to have a much lower iron requirement than reticulocytes, contain similar numbers of immunoreactive receptors per cell when in an active state of proliferation. Although receptor density on fibroblasts is directly related to cell proliferation, our studies demonstrate that nonproliferating fibroblasts still retain significant numbers of immunoreactive receptors and transferrin binding sites. Since additional studies indicate that proliferating cells have increased iron uptake, a simple hypothesis would predict that the parallel increase in transferrin binding sites and total cellular immunoreactive receptor associated with proliferation is related to an increased cellular iron requirement. However, the number of immunoreactive receptor molecules and transferrin binding sites is not changed when cells are grown in iron-deficient media, or in media with added transferrin-iron. This result and the lack of marked differences in receptor number on both hemoglobin-producing and nonhemoglobin-producing cells indicate that other factors besides receptor density play major roles in the regulation of cellular iron uptake, retention, and loss. (50 Refs)

12. ENHANCED TUMOR GROWTH IN VIVO BY A FACTOR IN HUMAN PLATELETS AND RAT LIVER.

Witkoski E, Kepner N, Leitzel K, Rogers C, Jefferson LS, Lipton A (c/o Lipton) Div. Oncology, M. S. Hershey Medical Center, Pennsylvania State Univ., Hershey, PA, 17033
Cancer Res; 42(6):2350-2352 1982

Liver cell supernatant and platelet extract can promote the in vivo growth of herpes simplex virus type 2-transformed hamster embryo fibroblast cells. Delineation of necessary growth factors for tumor cells in vivo may open up new strategies to inhibit malignant cell growth. (Author abstract) (15 Refs)

13. INHIBITION OF MOUSE NEUROBLASTOMA CELLS BY PROTEIN FACTOR DERIVED FROM RAT GLIOBLASTS.

Kato T, Sakazaki Y, Yamakawa Y, Kato H, Naganawa N, Kato T, Tsunooka H, Masaoka A, Tanaka R
Dept. Biochemistry (1st Div.), Nagoya City Univ. Medical Sch., Mizuho-ku, Nagoya 467, Japan
Dev Brain Res; 3(4):645-651 1982

A neuroblastoma growth-inhibition factor (NGIF), isolated from the medium of cultured fetal rat glioblasts, inhibited growth and DNA synthesis in cultured mouse neuroblastoma cells (Neuro-2a, NS-20Y, or N1E-115). NGIF promoted the morphologic differentiation of the mouse neuroblastoma cells but did not reduce their viability. NGIF did not increase intracellular cyclic AMP or cyclic guanosine monophosphate levels in the mouse neuroblastoma cells. At concentrations which significantly inhibited DNA synthesis in the mouse neuroblastoma cells, NGIF enhanced DNA synthesis in rat C6 glioma cells and had no effect in mouse neuroblastoma-like cells (Swiss 3T3 or 3T6) or rat glial cell lines LRM-55 and 354A. Exposure to NGIF did not affect the proliferation or morphologic differentiation of the glial cell lines, suggesting that under physiologic conditions it may act specifically on neuronal cells. High concentrations (greater than 25%) of fetal bovine serum in the medium abolished the inhibitory effects of NGIF on DNA synthesis in mouse neuroblastoma cells, suggesting that NGIF may be inactivated by serum soon after its migration to the bloodstream. The apparent mol wt (75,000), isoelectric point (5.8), absence of esterase activity, and biologic activities of NGIF differed from those of glial factor (isolated from C6 rat glioma) and gamma subunit of nerve growth factor. A/J mice inoculated with NGIF-exposed neuroblastoma cells survived longer than controls inoculated with untreated cells, although the times to palpable tumor appearance were about the same in the two groups. Daily injection of NGIF into the region adjacent to the tumor reduced tumor growth and significantly prolonged the survival time. (11 Refs)

14. REGULATION OF GRANULOCYTE AND MACROPHAGE COLONY-STIMULATING FACTOR PRODUCTION BY PHYTOHAEMAGGLUTININ-TREATED BLOOD MONONUCLEAR CELLS.

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Leuk Res; 6(1):71-79 1982

Medium conditioned by incubation of phytohemagglutinin (PHA: 25 ul/ml)-stimulated human peripheral blood mononuclear cells for 7 days was tested for colony-stimulating activity (CSA) for granulocyte-macrophage colony-forming cells from normal bone marrow. The conditioned medium gave two peaks of stimulation: one at a concentration of 5-10% (peak I) and the other at a concentration of 20% (peak II). Stimulation at 15% was considerably less. With stimulation by peak I material most of the colonies were neutrophilic on days 7 and 14. With peak II material few colonies were seen at 7 days, but at 14 days colonies contained macrophages or mixtures of macrophages and neutrophils. Removal of adherent cells prior to culture abrogated all stimulating activity of both peaks. However, supernatant of conditioned medium incubated with adherent cells for 1 wk ('reconditioned' medium) stimulated colony growth. PHA-stimulated mononuclear cells appear to act on adherent cells releasing colony-stimulating factor. (20 Refs)

15. ACUTE MYELOID LEUKEMIA COLONY GROWTH IN VITRO: DIFFERENCES OF COLONY-FORMING CELLS IN PHA-SUPPLEMENTED AND STANDARD LEUKOCYTE FEEDER CULTURES.

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Blood; 59(4):816-821 1982

Bone marrow or blood of patients with acute myeloid leukemia was subjected to cell separation and the cells investigated for in vitro colony growth. Discontinuous albumin density gradient centrifugation and depletion of E-rosette-forming cells resulted in purified fractions of acute myeloid leukemia cells. From these fractions, growth of large leukemic colonies was obtained in the PHA-leukocyte feeder (PHA-LF) colony tech-

nique in 12 of 14 patients. The standard double agar layer technique with a leukocyte feeder for granulocyte-macrophage colony forming cells (GM-CFC) supported colony formation in only four cases. The PHA-LF leukemic colony-forming cells (CFC) were found to be of low buoyant density (always less than or equal to 1.062 g/ml-1) when compared to normal marrow GM-CFC (peak at 1.065t g/ml-1). The density profile of PHA-LF CFC paralleled the distribution of the nucleated cells in eight cases, but in four patients, the CFC peak was found at a distinctly lower density; this suggested that a specific leukemic subpopulation had a colony-forming capacity. In three of of the four patients with colony growth in the double layer agar technique, it was evident that these CFC had density properties different from those of PHA-LF CFC. These findings suggest that cells giving rise to large colonies in the PHA-LF and double layer agar assays represent distinct leukemic subpopulations. (Author abstract) (24 Refs)

16. G-CSF: ITS RELATIONSHIP TO LEUKEMIA DIFFERENTIATION-INDUCING ACTIVITY AND OTHER HEMOPOIETIC REGULATORS.

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Dept. Developmental Hematopoiesis, Sloan-Kettering Inst. for Cancer Res., New York, NY, 10021
J Cell Physiol; Suppl1:53-64 1982

The interrelationship of the various factors produced by WEHI-3 leukemic cells was investigated with particular emphasis on the neutrophil-granulocyte colony-stimulating factor (G-CSF) activity and its relationship to leukemia differentiation-inducing activities induced by endotoxin in mouse and human sera. After stepwise column chromatography of WEHI-3D- conditioned medium (CM), the breakthrough fraction stimulated predominantly neutrophil-granulocyte colony formation, whereas the fraction eluted with 1 M NaCl induced primarily macrophage colony growth. The unseparated but concentrated CM contained significant mast cell growth factors and was a more active source than concanavalin A-stimulated mouse spleen CM. Tumor necrosis factor (TNF) according to the present study, is biochemically and functionally distinct from differentiation-inducing protein (GM-DF). The apparent lack of species specificity of TNF and its selective ability to inhibit proliferation of transformed cells, including WEHI-3B, suggests that acquisition of in vivo sensitivity of TNF may prove an important marker of leukemic transformation. The ability of Corynebacterium parvum plus endotoxin to induce high serum levels of TNF and of GM-DF with similar kinetics points to the importance of synergism studies with various biological response modifiers since TNF inhibits leukemic cell clonal expansion by a direct antiproliferative action, whereas GM-DF inhibits leukemic cell self-renewal by promoting terminal differentiation. (43 Refs)

17. HUMAN COLONY-STIMULATING FACTOR PRODUCING CELL LINE.

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Cell Mol Biol; 27(6):579-587 1981

Colony-stimulating factor (CSF) is considered to be a physiological granulopoietin which controls differentiation of precursor cells into granulocytes or macrophages. The studies on human CSF have been hampered by the difficulty in obtaining the material in large quantities for purification. In 1974, a human CSF-producing tumor was successfully transplanted into athymic nude mice and maintained by serial passages. Human CSF-producing tumors maintained in nude mice served as a constant source for the purification of human CSF, but its usefulness was limited by the small size of the tumors obtained from the nude mice. From these tumors grown in nude mice, human CSF-producing cell lines have been established, and large-scale production of human CSF has become possible by the use of the cultured medium of the cells. The partially purified CSF in large quantities may serve as a standard human CSF, thus facilitating the further extensive studies on human CSF. It is our goal that the human CSF may serve as a therapeutic drug against neutropenia and other hematological disorders such as myelogenous leukemia. (Author abstract) (46 Refs)

18. SOURCES AND BIOLOGY OF REGULATORY FACTORS ACTIVE ON MOUSE MYELOID LEUKEMIC CELLS.

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Cancer Res. Unit, Walter and Eliza Hall Inst. Medical Res., Royal Melbourne Hosp., Melbourne, 3050 Victoria, Australia
J Cell Physiol; Suppl1:175-183 1982

The cell types producing granulocyte-colony stimulating factor (G-CSF), the half-life of G-CSF in the serum, and the effects of endotoxin in modifying serum G-CSF levels were studied. Mice used were 3-mo-old male and female mice of the strains C57BL and BALB/c. Following a single iv injection of 5 ug endotoxin, the capacity of the serum to induce differentiation colonies of WEHI-3B cells rises rapidly to a peak at 3-6 hr. Groups of C57BL mice were injected iv with 5 ug endotoxin 4x/wk. Good responses were obtained following the first three injections but thereafter successive responses were weaker and after the seventh injection no significant rises in activity were observed. Irradiation (150-1,200 rad) did not prevent the mice from responding to a subsequent injection immediately after irradiation. Groups of BALB/c nu/nu (congenitally athymic) mice were injected with the same agent. Nu/nu mice exhibited a normal response to the injection and exhibited obvious elevations in serum differentiating activity. Effects of cocultivation of WEHI-3B cells with normal cells were studied. Neither normal BALB/c thymus nor peritoneal cells formed colonies in the type of agar medium used. Addition of thymus cells did not induce differentiation in WEHI-3B colonies. Addition of 10(6) BALB/c peritoneal cells caused a marked differentiation of WEHI-3B colonies. Serum differentiating activity half-life varied between 1.5 and 2.3 hr. Groups of BALB/c mice were sc injected with 10(6) WEHI-3B cells. Recipient mice developed sc tumors within 3-4 wk of transplantation. No elevated levels of differentiating activity were observed. (41 Refs)

19. EFFECTS OF CELL CONCENTRATIONS ON THE SURVIVAL AND REPOPULATION OF HAEMOPOIETIC STEM CELLS IN IRRADIATED BONE MARROW CELL CULTURE IN VIVO.

Fujitake H, Okamoto Y, Okuba H, Miyanomae T, Kumagai K, Mori KJ
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J Radiat Res (Tokyo); 22(4):449-455 1981

Bone marrow cells from a pathogen-free male mice of DDY-strain were cultured, subjected to 200 R of x-rays, and assayed for survival of CFUs and of colony forming unit (CFU) culture (c) or stem (s) cells. There was no difference in survival of stem cells, either CFUs or CFUc at the concentration of 10(5)-

10(6) cells/ml when determined immediately after exposure to irradiation. At 1 wk after irradiation, control cells had similar numbers of CFUs, CFUc, and total cells in suspension, regardless of the concentration of bone marrow cells. In low density cultures, however, CFUs increased significantly from the levels immediately after irradiation, but in high-density cultures no such recovery was apparent. At 3 wk after irradiation a substantial increase in CFUs had occurred in high density cultures, but less than that in low density cultures. The number of CFUc per 10(5) cells was less in low density cultures than in high density cultures at this time. Since long-term culture of murine bone marrow cells is not affected by contact inhibition of the cells involved in hemopoiesis, it is concluded that CFUs proliferation in this system is at least partly controlled by end product inhibition, and that mature cells in the hemopoietic tissues are responsible for production of the factor. (24 Refs)

20. REGULATION OF PLURIPOTENT STEM CELL PROLIFERATION AND DIFFERENTIATION: THE ROLE OF LONG-RANGE HUMORAL FACTORS.

Tubiana M, Frindel E
Institut de Radiobiologie Clinique, Institut Gustave-Roussy, 94800 Villejuif, France
J Cell Physiol; Suppl1:13-21 1982

Stimulation of quiescent pluripotent stem cell colony forming units (CFU-S), inhibition of CFU-S proliferation, and differentiation pathways of CFU-S are discussed. The proliferative status of the stem cells of mouse bone marrow was studied after partial body irradiation and selective killing of maturing hemopoietic cells. Data on the feedback mechanism between different hemopoietic areas, between the differentiated cells and the stem cell compartments, and within the stem cell compartments itself were obtained. (58 Refs)

21. CONTROL GROWTH AND NORMAL DIFFERENTIATION IN LEUKEMIC CELLS: REGULATION OF THE DEVELOPMENTAL PROGRAM AND RESTORATION OF THE NORMAL PHENOTYPE IN MYELOID LEUKEMIA.

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Dept. Genetics, Weizmann Inst. Science, Rehovot 76100, Israel
J Cell Physiol; Suppl1:151-164 1982

Restoration of the normal phenotype in myeloid leukemia (ML) by induction of normal differentiation by the physiological inducer of differentiation; macrophage and granulocyte inducing (MGI) proteins and the regulation of growth and differentiation in normal and leukemic myeloid cells; uncoupling of controls for growth and differentiation; constitutive gene expression, the normal developmental program, and the origin and evolution of ML; the role of different molecular forms of macrophage and granulocyte inducer; induction of differentiation by treatment with other compounds; and developmental programs and malignancy in different cell types are discussed. Normal myeloid precursor cells cultured with a feeder layer of other cell types can form colonies of granulocytes and macrophages in vitro. Proliferation in the normal cells is regulated at two control points. The first control is that which requires MGI to produce more cells that can then differentiate, and the second control is the stopping of cell multiplication that occurs in the program of the terminal differentiation in the mature cells. Since the normal but not the leukemic myeloblasts require MGI for cell viability and proliferation, the molecular changes required for cell viability and proliferation that have to be induced in the normal cells are constitutive in the leukemic cells. The MGI independent proliferation of ML cells appears to proceed through stages, probably starting from a small decrease to finally a complete loss of the requirement for MGI for cell viability and proliferation. ML cells show a clonal difference in inducibility for differentiation by the physiological inducer MGI-2. ML cells also show a clonal difference in inducibility for differentiation by steroid hormones, nonphysiological inducers, and by compounds that enhanced the effect of some inducing compounds. (90 Refs)

22. MODULATION OF CELL COMPETENCE FOR INDUCTION OF DIFFERENTIATION IN MYELOID LEUKEMIC CELLS.

Symonds G, Sachs L
Dept. Genetics, Weizmann Inst. Sciences, Rehovot 76100, Israel
J Cell Physiol; 111(1):9-14 1982

Physiological ways of modulating cell competence for induction of differentiation by a variety of compounds were investigated. Cell competence for induction of some differentiation-associated properties in differentiation-defective clones of mouse myeloid leukemic cells (MGI 7-1D 7-1) could be modulated for some inducers [macrophage-granulocyte inducing protein (MGI-2), lipopolysaccharide (LPS), and dexamethasone (DEX)] by the type of serum in the medium and for another inducer (insulin) by removing serum from the medium. The molecular of MGI that induced this differentiation was MGI-2. All MGI 7-1D 7-1 clones could be modulated for inducibility by MGI-2, LPS, and DEX, but only some of these clones could be modulated for inducibility by insulin. These modulations allowed induction of some but not all differentiation-associated properties, so that both procedures modulated in MGI 7-1D 7-1 clones the expression of some but not all the genes in the developmental program. (33 Refs)

23. EFFECT OF VARIOUS IMMUNOREGULATORY SUBSTANCES ON THE GROWTH INHIBITORY ACTIVITY (GIA) OF HUMAN MONOCYTES AGAINST LYMPHOBLASTOID CELL LINES.

Jett JR, Hoffman TM, Connor RJ, Herberman RB
NCI, NIH, Building 10/8B11, Bethesda, MD, 20205
Immunopharmacology; 4(2):105-113 1982

Environmental or disease-related factors that may influence levels of cytostatic activity were studied and pharmacological manipulations were performed in an attempt to elucidate mechanisms involved in monocyte-tumor reaction and regulation. Human monocytes from healthy individuals are able to inhibit the growth, in vitro, of certain transformed lines. The activity was augmented by phorbol-12-myristate-13-acetate, and to some extent, interferon, but was not measurably affected by superoxide dismutase, catalase, prostaglandins, or steroids. Growth inhibitory activity was manifested by highly purified monocyte populations, whether obtained by elutriation or by removal after adherence to plastic. (23 Refs)

24. SOLUBLE CYTOSTATIC FACTOR(S) RELEASED FROM HUMAN MONOCYTES. I. PRODUCTION AND EFFECT ON NORMAL AND TRANSFORMED HUMAN TARGET CELLS.

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Section Haematology and Immunology, Dept. Medicine, Trondheim Regional Hosp., N-7000 Trondheim, Norway
Scand J Immunol; 15(3):311-318 1982

Human monocytes activated with lymphokines after 3-7 days of in vitro maturation released stable cytostatic factor(s) (CF), as determined by reduction of [methyl-3H]thymidine incorporation and cell counts in target cell cultures. Lipopolysaccharide from Escherichia coli slightly enhanced the release of CF. The CF release was greatest at an intermediate stage of the in vitro monocyte maturation to large, macrophage-like cells. Three transformed human cell lines (NHIK 3025, K-562, and U-20S) and normal skin fibroblasts were consistently inhibited by CF. Normal human mesothelial cells were variably affected, while normal human lymphocytes proliferating in response to allogeneic cells in mixed lymphocyte culture were not inhibited. Further analysis of CF may disclose one of the mechanisms whereby human monocytes and macrophages inhibit growth of human tumor cells. (Author abstract) (34 Refs)

25. STEM CELL (CFUS) PROLIFERATION INHIBITOR IN BLOOD OF MICE INJECTED WITH HYDROXYUREA.

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Dept. Pathophysiology, Charles Univ., U nemocnice 5, 128 53 Praha 2, Czechoslovakia
Cell Tissue Kinet; 15(3):263-268 1982

Analysis of the mouse hemopoietic stem cell (CFUs) kinetics after hydroxyurea administration has provided an in vivo assay suitable for detection of factors which inhibit recruitment of non-proliferating G0-CFUs into cell cycle, or transit of CFU's through the G1 phase. Using this assay, it has been demonstrated that plasma obtained from mice which had received hydroxyurea approx 12-14 hr previously, possesses a factor which inhibited the triggering of CFUs into the cell cycle. The appearance of this CFUs proliferation inhibitor occurred at a time when 60-70% of the CFUs were synchronized in the S phase of the cell cycle, as a consequence of hydroxyurea action. Some basic properties of the inhibitor were investigated. (Author abstract) (10 Refs)

26. INTERLEUKIN 1, A POTENTIAL REGULATOR OF FIBROBLAST PROLIFERATION.

Schmidt JA, Mizel SB, Cohen D, Green I
Lab. Immunology, Natl. Inst. Allergy and Infectious Diseases, NIH, Bethesda, MD, 20205
J Immunol; 128(5):2177-2182 1982

Secondary mixed lymphocyte reaction (MLR) supernatants contain factors that stimulate fibroblast proliferation in vitro. Biochemical analysis of secondary MLR supernatants using gel filtration chromatography and isoelectrofocusing (IEF) demonstrated that the fibroblast proliferation factors have apparent mol wt of 75,000 and 13,000 and isoelectric points (pI) of 6.8 to 7.2, 5.8 to 6.2, and 5.2 to 5.4, values similar to those previously reported for human interleukin 1 (IL 1). To further explore the relationship between the MLR-derived fibroblast proliferation factors and IL 1, the following experiments were performed. First, the fractions obtained from gel filtration and IEF were assayed for both thymocyte proliferation activity, a measure of IL 1 activity, and fibroblast proliferation activity, and superimposable activity profiles were obtained. Second, to confirm that the observed thymocyte proliferation activity was in fact due to IL 1 and not interleukin 2 (IL 2) or novel factors, the IEF fractions contained peak thymocyte and fibroblast proliferation activity (ie, fractions having pI of 6.8, 6.0, and 5.3, respectively) were demonstrated to stimulate IL 2 production by a murine T lymphoma line, a specific measure of IL 1 activity. Third, IL 1 and the fibroblast proliferation factors were co-purified through a three-step, 200-plus-fold purification protocol consisting of ammonium sulfate precipitation, gel filtration chromatography, and IEF. Fourth partially purified human IL 1 made in another laboratory and highly purified murine IL 1, but not human and murine IL 2, stimulated fibroblast proliferation. These results demonstrate that the fibroblast proliferation factors found in secondary MLR supernatants have several functional and biochemical properties in common with human IL 1. These findings raise the possibility that IL 1 may regulate fibroblast proliferation and that the local production of IL 1 by infiltrating mononuclear cells may contribute to the fibrosis observed in chronic inflammatory disease states. (Author abstract) (55 Refs)

27. MONOCLONAL HUMAN B LYMPHOMA CELLS RESPOND BY DNA SYNTHESIS TO ANTI-IMMUNOGLOBULINS IN THE PRESENCE OF THE TUMOUR PROMOTOR TPA.

Godal T, Henriksen AA, Ruud E, Michaelsen T
Lab. Immunology, The Norwegian Radium Hosp., Montebello, Oslo 3, Norway
Scand J Immunol; 15(3):267-274 1982

Monoclonal human B lymphoma cells were triggered in vitro to DNA synthesis with F(ab')2 fragments of rabbit IgG antibodies specific for different human immunoglobulin (Ig) light and heavy chains. The specificity of the responses corresponded to surface Ig (sIg) present on tumor cells. Anti-mu chain, anti-delta chain, anti-gamma chain, and antibodies to light chains were found to mediate this effect. However, to induce DNA synthesis, the tumor promotor 12-O-tetradecanoyl-phorbol-13-acetate (TPA) was usually required. TPA alone induced cytoplasmic protrusions and increases in cellular volume. These observations should provide new opportunities to study the triggering of B cells with anti-Ig in well-defined cell populations. (Author abstract) (33 Refs)

28. A DIFFERENTIATION FACTOR REQUIRED FOR THE EXPRESSION OF CYTOTOXIC T-CELL FUNCTION.

Raulet DH, Bevan MJ
Dept. Pathology, Univ. Pennsylvania, Philadelphia, PA, 19104
Nature; 296(5859):754-757 1982

Activation of cytotoxic T lymphocytes (CTL) was examined. CTL polyclonal induction from T cell populations was mediated by concanavalin A (Con A). The responding T-cell population was depleted of accessory cells; Con A-induced proliferation and differentiation were thus dependent on exogenous factors. Activation was shown to involve separate growth-inducing and differentiation-inducing activities: interleukin-2 (IL-2) and CTL differentiation factor (CDF), respectively. The molecular independence of IL-2 and CDF were demonstrated. (33 Refs)

29. RECEPTORS FOR T-CELL GROWTH.

Kipp DE, Askonas BA
Immunology Div., MRC Natl. Inst. Medical Res., Mill Hill, London NW7 1AA, England
Nature; 296(5859):707 1982

T-cell growth factors (TCGF) and their biological significance are discussed. While the resting T-cell is unresponsive to TCGF, mitogen- or antigen-activated T-cells do respond. All of the non-T-cell human neoplastic cell lines and 90% of human T-cell human neoplastic cell lines studied have failed to bind TCGF in vitro. Transformed cell lines may have lost expression of TCGF receptors or may have been derived from early T-cells that had not yet expressed receptor activity. (10 Refs)

30. INTERLEUKIN 3: POSSIBLE ROLES IN THE REGULATION OF LYMPHOCYTE DIFFERENTIATION AND GROWTH.

Ihle JN, Rebar L, Keller J, Lee JC, Hapel AJ
Frederick Cancer Res. Facility, P. O. Box B, Frederick, MD, 21701
Immunol Rev; 63:5-32 1982

Interleukin-3 appears to primarily influence immature lymphocyte differentiation, unlike interleukin-2. The role of 20 alpha hydroxysteroid dehydrogenase in lymphocyte differentiation is also discussed, as are the biochemical and biological characteristics of interleukin-3. (39 Refs)

31. INHIBITION OF THE LYMPHOCYTE BLASTOGENIC RESPONSE TO ANTIGEN BY SERUM-FREE CULTURE SUPERNATANTS OF LEUKEMIC B CELLS.

Walsh JD, Geczy CL
Kolling Inst. Medical Res., Royal North Shore Hosp. Sydney, New South Wales 2065, Australia
Cell Immunol; 67(2):241-254 1982

Suppressive factors were detected in culture supernatants of the guinea pig B-cell L2C leukemia. Dialyzed culture supernatants (DCS) inhibited the blastogenic response of sensitized lymph node cells (LNC) to a wide dose range of the sensitizing antigen (ovalbumin or PPD) but failed to inhibit the proliferative response to PHA or Con A. In addition, DCS inhibited the response of blast cells to preformed T-cell growth factor (TCGF). The inhibitor(s) in DCS was noncytotoxic, heat stable (30 min at 80 C), resistant to treatment with trypsin, and exerted its effect subsequent to activation of sensitized LNC by antigen. Washing of DCS-treated cells restored normal reactivity to a subsequent antigen challenge. The target cell for the inhibitor may be cells responding to amplification signals produced by activated T cells. KCl (3 M) extracts of L2C cells behaved like DCS in inhibiting only antigen responses. Both undialyzed culture supernatants (UCS) and leukemic sera inhibited mitogen, allogeneic, and antigen-stimulated proliferative responses by greater than 80%. These soluble factors may participate in the depression of cell-mediated immunity associated with lymphoid malignancies. (Author abstract) (36 Refs)

32. ENHANCED T CELL SUPPRESSION IS DIRECTED TOWARD SENSITIVE CIRCULATING B CELLS IN MULTIPLE MYELOMA.

Perri RT, Oken MM, Kay NE
Dept. Medicine, Hematology Section (111E), Veterans Admin. Medical Center, 54th St. and 48th Ave. South, Minneapolis, MN, 55417
J Lab Clin Med; 99(4):512-519 1982

Multiple myeloma, a disorder typically accompanied by monoclonal immunoglobulin excess and hypogammaglobulinemia was evaluated to delineate the status of T cell regulation on malignant B cell proliferation. Intact T cells and T cell subsets previously noted by us to be suppressor (Tgamma) and helper (T non-gamma) were isolated from 10 controls and 10 myeloma patients. Various concentrations of control and patient T cell subsets were mixed with control and patient B cells and stimulated with PWM. T cell effect on B cell proliferation as measured by [3H]thymidine incorporation was then determined. Myeloma Tgamma cells were more effective than control Tgamma cells in suppressing control or myeloma B cell [3H]thymidine incorporation. Interestingly, myeloma B cells, when added to myeloma Tgamma cells, were suppressed to a greater extent than were control B cells when mixed with a similar concentration of myeloma Tgamma cells. These in vitro studies suggest a complexity of B and T cell abnormalities in multiple myeloma; first, the myeloma B cells are extremely sensitive to Tgamma cell suppression, and second, myeloma Tgamma cells have excessive suppressor capacity. It is yet unclear whether these results are associated with an immunoregulatory response to this malignancy or represent part of the basic disease process. (Author abstract) (22 Refs)

33. MULLERIAN INHIBITING SUBSTANCE INHIBITS COLONY GROWTH OF A HUMAN OVARIAN CARCINOMA CELL LINE.

Fuller AF, Guy S, Budzik GP, Donahoe PK
(c/o Donohoe) Pediatric Surgical Res. Lab., Massachusetts General Hosp., Boston, MA, 02114
J Clin Endocrinol Metab; 54(5):1051-1055 1982

Mullerian inhibiting substance (MIS), a fetal testicular product which causes regression of the Mullerian duct in the male mammalian embryo, is being evaluated as an inhibitor of genital tract tumors. The present study demonstrates in a clonogenic soft agar assay that biologically active MIS inhibits the growth of a human ovarian carcinoma cell line (HOC-21) in a dose-dependent manner when compared to phosphate-buffered saline medium controls, heat-inactivated MIS, or biochemical fractions which lack biological activity (p less than 0.05). (Author abstract) (13 Refs)

34. THE IDENTIFICATION OF A SERUM VIABILITY FACTOR FOR SV3T3 CELLS AS BIOTIN AND ITS POSSIBLE RELATIONSHIP TO THE MAINTENANCE OF KREBS CYCLE ACTIVITY.

Nakano ET, Ciampi NA, Young DV
Dept. Chemistry, Boston Univ., 685 Commonwealth Ave., Boston, MA, 02215
Arch Biochem Biophys; 215(2):356-563 1982

A factor isolated from calf serum using Sephadex G-25 gel filtration enhanced the viability and growth of SV40-transformed mouse 3T3 fibroblasts (SV3T3) grown in low-serum medium. This factor (peak III) was identified as biotin, based on the following observations: (1) peak III exhibited biological effects similar to biotin, eg reduction in the number of dead SV3T3 cells in culture, reduction in amount of lactic acid released by these cells, and a synergism with iron in stimulating growth; (2) following passage of peak III through an avidin-Sepharose affinity column, no viability/growth activity for SV3T3 was detected in the eluted fractions, but the activity was completely recovered following passage of peak III through a column of Sepharose alone; (3) peak III extracts reacted with p-dimethylaminocinnamaldehyde, a reagent which complexes with the cyclic ureide moiety of biotin; and (4) peak III exhibited the same relative front as authentic biotin on thin

layer chromatography. When individual Krebs cycle intermediates were assayed for their ability to stimulate SV3T3 proliferation in low-serum medium not supplemented with biotin, malate, citrate, isocitrate, and fumarate were growth promoting, while alpha-ketoglutarate, oxaloacetate, and succinate were ineffective. These results are consistent with the hypothesis that biotin exerts its viability/growth promoting ability via cofactor activity with pyruvate carboxylase, thereby helping to maintain the levels of Krebs cycle intermediates. (20 Refs)

35. GROWTH CONTROL IN CULTURED 3T3 FIBROBLASTS. III. BINDING INTERACTIONS OF A GROWTH INHIBITORY ACTIVITY WITH TARGET CELLS.

Voss PG, Steck PA, Calamia JC, Wang JL
Dept. Biochemistry, Michigan State Univ., East Lansing, MI, 48824
Exp Cell Res; 138(2):397-407 1982

A Fibroblast Growth Regulator (FGR), which inhibits the growth and division of proliferating fibroblasts, has been isolated from medium conditioned by exposure to density-inhibited 3T3 cells. The binding of this FGR to its target cells has been analyzed using [35S]methionine-labeled inhibitor. The key features of this interaction are (a) there are approx 3-4 x 10(5) binding sites/cell; (b) more ligand is bound at 37 than at 4 C, possibly due to internalization of the molecule at the higher temperature; (c) on a per cell basis, approx the same amount of ligand is bound at high cell density as at low cell density; (d) there is no evidence of target cell heterogeneity in the binding of the inhibitor; and (e) the binding can be inhibited partially by calf serum. The results suggest that modulation of cell surface receptor binding by serum and growth inhibitory factors may be an important feature in growth regulation. (Author abstract) (23 Refs)

36. SELF-NORMALIZATION OF HIGHLY TRANSFORMED 3T3 CELLS THROUGH MAXIMIZED CONTACT INTERACTION.

Rubin H, Chu BM
Dept. Molecular Biology, Univ. California, Berkeley, CA, 94720
Proc Natl Acad Sci USA; 79(6,part1):1903-1907 1982

Nontransformed and moderately and highly transformed BALB/c 3T3 cells maintained on small coverslips in a large vol of medium multiplied to 2, 3, and 4x higher population density, respectively, than they did in conventional cultures. Deprivation of Mg++ caused highly transformed cells on coverslips to assume the appearance of nontransformed cells, decrease their rate of multiplication, and stop further growth at a much lower saturation density than the same cells in physiological Mg++. The latter cells reached a saturation density of 10(6)/cm2 and their rate of DNA synthesis decreased progressively with increased crowding. At saturation density, cells in physiological Mg++ took on an appearance and arrangement similar to normal fibroblasts. They developed a high requirement for serum to initiate DNA synthesis. When transferred at low density, they flattened out on a plastic surface and maintained the appearance of nontransformed cells for approx 1 day. Onset of DNA synthesis and multiplication in the transferred cells was delayed for periods characteristic of quiescent nontransformed cells stimulated by fresh medium or transfer. Cells from crowded coverslips were approx 1/10th as efficient at colony formation when suspended in agar as cells from uncrowded coverslips. They also had a significantly lower Mg++ content. The crowded cells returned to their transformed morphological and growth behavior 2 to 3 days after transfer at low density. We conclude that a very high degree of crowding causes highly transformed cells to revert to the phenotype of non-transformed cells. Other treatments such as deprivation of Mg++ or inorganic orthophosphate can achieve similar results. It appears that a balanced reduction in rates of metabolism and multiplication can restore the normal phenotype to transformed cells, implying that they differ only quantitatively from nontransformed cells. The putative role of Mg++ in the regulation of multiplication and in transformation of animal cells is discussed. (Author abstract) (17 Refs)

37. DIFFERENTIATION OF EHRLICH'S ASCITES CARCINOMA CELLS CULTURED IN VITRO.

Shuklinov VA, Shumilina VV
R. E. Kavetskii Inst. Oncology Problems, Kiev, USSR
Dokl Akad Nauk SSSR; 262(1):222-225 1982

The differentiation of Ehrlich's ascites carcinoma cells was studied in long-term monolayer cultures of murine fibroblasts in the presence of 60% bovine serum. Cell differentiation of cytological and histological type was observed despite the long existence of the cell line. The cell differentiation was proceeding toward the original murine mammary gland tissue. The findings indicate that the determination of the cells is preserved despite transformation to cancer cells, but differentiation required high serum concentration. (6 Refs) (In RUS)

38. GENE CONTROLLED NEGATIVE REGULATION OF DNA SYNTHESIS IN ERYTHROPOIETIC PROGENITOR CELLS.

Axelrad A, Croizat H, Eskinazi D, Stewart S, Vaithilingam D, Van der Gaag H
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J Cell Physiol; Suppl1:165-173 1982

Negative regulation of DNA synthesis in early erythropoietic progenitor cells (BFU-E) and its relation to Friend polycythemia virus (FV) are discussed. Evidence for the negative regulatory activity in B6 mice marrow cells is presented. Results indicate that the activity is under strict control. The activity is detectable at approx the same low concentration at which it is found in B6 marrow, as shown by the rapidity with which it dilutes out in titration curves. The activity appears to be specific for the BFU-E population and is readily reversible without apparent toxicity. No reduction in burst-forming efficiency was observed, and the proportion in DNA synthesis is not reduced by the cells having been in contact with B6 marrow supernatant. Evidence for operation of the negative regulator (NR) on the cell surface is presented. NR is readily removed by a single wash and is immediately effective when added to the cells although its mol wt appears to be high, it is accessible to antibody while associated with the cells, and it functions at 0 C. The NR of BFU-E DNA synthesis was discovered in normal B6 mice during the course of investigations of the mechanism by which the Fv-2rr genotype confers solid resistance against the development of FV-induced polycythemia in adult mice. Effects of B6 NR of BFU-E DNA synthesis on amplification of megakaryocyte colony forming units (CFU-E) by FV and erythropoietin were studied in vitro. Results indicate that inclusion of concentrated B6 marrow supernatant in the liquid cultures entirely prevented the amplification of the CFU-E population brought about by FV and erythropoietin. (28 Refs)

39. STUDIES ON THE ANTITUMOR ACTIVITY OF FUSOBACTERIUM NUCLEATUM STRAIN KO-31.

Tamai K, Nakao J, Takematsu K, Nakagawa K
Clinic Dento-Oral Surgery, Kanazawa Univ. Sch. Medicine, Kanazawa, Ishikawa 920, Japan
Microbiol Immunol; 26(2):163-165 1982

The antitumor activity of a culture supernatant fluid of Fusobacterium nucleatum strain KO-31 was investigated in ICR mice bearing Ehrlich ascites carcinoma. Mice were injected with a carcinoma cell suspension containing 8.0-12.0 x 10(6) cells/ml ip. Beginning 2 days later, a 0.5 ml dose of test sample was given ip daily for 7 days. The mice were observed for 60 days and the antitumor activity [expressed as T/C(%) = (survival days of treated group divided by survival days of control group) x 100] was determined. The T/C(%) of mice treated with culture supernatant fluid was 206.7, compared with 103.0 for control mice. In several repeats of the same experiment, greater than 80% of mice inoculated with cancer cells survived when treated with the culture supernatant fluid. Dialysis or heating at 100 C for 10 min had no effect on the antitumor activity of the culture supernatant fluid. Cell extract from the same strain of F nucleatum showed no antitumor effect in mice bearing carcinoma. The antitumor substance may be a kind of lipopolysaccharide rather than a protein, evidenced by its heat stability. (2 Refs)

HORMONES/ STEROIDS/ PROSTAGLANDINS AS GROWTH EFFECTORS

40. CLONAL VARIANTS OF A MELANOMA CELL LINE SENSITIVE TO GROWTH INHIBITION BY DEXAMETHASONE.

Buzard RL, Hutchens TW, Hawkins EF, Markland FS, Horn D
Molecular Biology Div., Univ. Southern California, Los Angeles, CA, 90007
Exp Cell Res; 138(2):385-396 1982

We have isolated and characterized two clones of the RPMI 3460 Syrian hamster melanoma cell line which exhibit different responses to the synthetic glucocorticoid dexamethasone. In the presence of 10 nM dexamethasone, one clone (Clone 6) exhibits the growth inhibition, morphological alterations, and reduction in final cell density observed in the parental RPMI 3460 cell line. In contrast, the other clone (Clone 5), although exhibiting a reduction in final cell density, fails to exhibit the growth inhibition and morphological alterations. Thus, the effect of dexamethasone on growth and morphology can be expressed separately from the effect of dexamethasone on final cell density in these cells. This observation suggests that the two sets of responses can be controlled separately and that glucocorticoids may exert their influence through different or divergent biological pathways. In vitro receptor assays suggest that the different phenotypes of Clone 5 compared with Clone 6 cells cannot be explained by an absence of or reduction in cytosolic glucocorticoid receptor, in Clone 5 cells. Additional receptor characterization suggests that the different responses to dexamethasone of Clone 5 and Clone 6 cells do not reflect changes in the ability of receptor to exist in a stably activated form. Differences in the accumulation or depletion of extracellular components in the growth medium also do not seem to be responsible for the altered phenotype of Clone 5 vis-a-vis Clone 6 cells. (Author abstract) (42 Refs)

41. STEROID HORMONE RECEPTORS AND THE GROWTH OF UTERINE MYOMA.

Vasilevskaia LN, Bassalyk LS, Fuks MA, Kuz'mina ZV, Murav'eva NI
Dept. Obstetrics and Gynecology, N. A. Semashko Medical Stomatological Inst., Moscow, USSR
Akush Ginekol (Mosk); (2):46-49 1982

Relationship between the concentration of steroid hormone receptors and the type of tumor growth was studied in 57 women (28-52 yr old) with uterine myoma. All patients were subjected to surgery: 41 underwent supravaginal amputation of the uterus, 11 underwent extirpation of the uterus, and 5 underwent conservative myomectomy. Indications for surgery included rapid growth of myoma, submucous localization of the myoma nodules, and uterine hemorrhage. Morphological examination of surgery specimens showed stable size of the myoma in 19 patients (Group 1), marked proliferation of smooth muscles or true growth of myoma in 28 (Group 2), and secondary changes associated with edema or dystrophy of myoma nodules in 10 (Group 3). Regardless of the type of myoma growth, concentration of estradiol receptors in the myoma nodule was almost 2x greater than that in the myometrium. Relationship between the concentration of progesterone receptors (PR) in the myoma tissue and in the myometrium depended upon the type of tumor growth: PR concentration in myoma tissue was similar to that in the myometrium in Group 1, exceeded that in the myometrium in Group 2, and was lower than that in the myometrium in Group 3. (10 Refs) (In RUS)

42. DNA REPLICATION AND PRODUCTION OF RETROVIRAL MARKERS IN HUMAN MELANOMA CULTURES DEPENDS ON DEGREE OF DIFFERENTIATION AND ON HORMONAL STIMULATION.

Van Tieghem N, Vercammen-Grandjean A, Fooij M, Temmerman A, Lejeune FJ
Laboratoire de Microbiology, Universite Libre de Bruxelles, Faculte de Medecine, Brussels, Belgium
Protides Biol Fluid Proc Colloq; 29:559-562 1982

Human melanoma cells in early passage and established cell lines from metastatic tumors were studied for growth, morphology, differentiation, and retroviral markers in the presence of alpha-melanin stimulating hormone (aMSH). aMSH inhibited cell number and DNA synthesis in cell line HM6A; no growth enhancement was observed in any of the cell lines. Dedifferentiated cells were more susceptible to the increased syncytia-forming effects of aMSH than other cell types. Cell counts of aMSH-exposed cells were generally inhibited due to production of toxic compounds during the pigmentation process. (9 Refs)

43. HORMONE-DEPENDENT TERMINAL DIFFERENTIATION IN VITRO OF CHICKEN ERYTHROLEUKEMIA CELLS TRANSFORMED BY TS MUTANTS OF AVIAN ERYTHROBLASTOSIS VIRUS.

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Inst. Virus Res., German Cancer Res. Center, Im Neuenheimer Feld 280, D-6900 Heidelberg, W. Germany
Cell; 28(4):907-919 1982

Chicken erythroblast cell strains and a cell line transformed by ts mutants of avian erythroblastosis virus (AEV) terminally differentiate when shifted to the nonpermissive temperature (42 C). The differentiated cells resemble mature erythrocytes with respect to morphology and ultrastructure, expression of differ-

entiation-specific cell-surface antigens, pattern of protein synthesis and hemoglobin content. Terminal differentiation is dependent on conditions favoring the differentiation of normal erythroid progenitor cells, including an erythropoietin-like factor. Colonies of ts AEV cells grown at 42 C in semisolid medium resemble erythrocyte colonies derived from normal erythroid progenitor cells. The colonies obtained were comparable in size or slightly larger than the late erythroid precursor (CFU-E) colonies. These results suggest that AEV-transformed cells are blocked at a stage of differentiation that is more advanced than that of the uninfected target cells. ts AEV cells are irreversibly committed to terminal differentiation within 20 to 30 hr after shift to 42 C. (Author abstract) (42 Refs)

44. GROWTH AND CELL SURVIVAL FOLLOWING TREATMENT WITH ESTRAMUSTINE, NOR-NITROGEN MUSTARD, ESTRADIOL AND TESTOSTERONE OF A HUMAN PROSTATIC CANCER CELL LINE.

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A B Leo Res. Lab., Box 941, S-251 09 Helsingborg, Sweden
J Urol; 127(4):818-822 1982

Estramustine at concentrations ranging from 3-40 x 10(-6) M inhibited the cell growth and clonogenic survival of a human prostatic carcinoma cell line (DU 145). This cell line was found to be unresponsive to estradiol and testosterone at concentrations ranging from 10(-9) M to 5 x 10(-5) M. Metabolism studies with estramustine showed that only a few per cent of the ester linkage was cleaved during the exposure period. This small amount of metabolism could possibly lead to the release of nor-nitrogen mustard, which was however found not to be as inhibitory as estramustine in this cell line. The results indicate that estramustine per se causes the cell death of hormone unresponsive human prostatic carcinoma cells in cell culture. (Author abstract) (28 Refs)

45. SERUM-FREE CULTURE OF RESTING, PHA-STIMULATED, AND TRANSFORMED LYMPHOID CELLS, INCLUDING HYBRIDOMAS.

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Exp Cell Res; 138(2):287-295 1982

A chemically-defined medium containing casein, insulin, testosterone, transferrin, and linoleic acid (CITTL) has been developed for the culture of lymphoid cells. Resting human mononuclear leukocytes can be maintained in CITTL for up to 8 wk with excellent viability. In the absence of mitogen, undifferentiated monocytes become the predominant cell type by 7-11 days as assessed by morphology, population distribution of cell sizes, and fluorescence-activated cell sorter analysis. Phytohemagglutinin (PHA)-induced proliferation is retained in these cultures for 7-10 days and results in a population of 85% T lymphoblasts. T lymphoblasts survive for 2 wk in CITTL following mitogenic stimulation and proliferate for up to 5 wk in CITTL if supplemented with 2-mercaptoethanol and conditioned medium containing crude T-cell growth factor. In addition to supporting proliferation of normal lymphoid cells, CITTL also sustains growth of human and murine hybridomas, as well as a variety of transformed lymphoid cell lines. The ability of a minimum essential medium supplemented with five or six factors to support growth of normal and malignant lymphoid cells suggests that only a limited number of extracellular signals are required for mitogenesis of these cells. (Author abstract) (49 Refs)

46. ENHANCEMENT OF HUMAN B LYMPHOCYTE DIFFERENTIATION IN VITRO BY THYROID HORMONE.

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Scand J Immunol; 15(2):211-215 1982

A study was made of the regulatory influence of a thyroid hormone, triiodothyronine (T3), on human blood T- and B-lymphocyte responses in vitro. Blood lymphocytes were stimulated with the T-cell mitogens concanavalin A (Con A) and phytohemagglutinin (PHA), with the T-dependent B-cell activator pokeweed mitogen (PWM), and with the T-independent B-cell activator Staphylococcus aureus Cowan I bacteria in the presence of various hormone concentrations. T3 did not stimulate T-cell proliferation. The number of immunoglobulin-containing and -secreting cells (plasmablasts) was increased in PWM and Staph aureus cultures treated with T3. The maximal enhancement was reached at a concentration of 10(-9) -10(-7) M T3. Cell fractionation techniques revealed that T3 apparently had a direct stimulatory effect on B-cell differentiation. (Author abstract) (18 Refs)

47. PROSTAGLANDIN SYNTHESIS AND BINDING IS INCREASED IN REGRESSING NMU MAMMARY CARCINOMA.

Liu SC, Knazek RA
Lab. Pathophysiology, NCI, NIH, Bethesda, MD
Prostaglandins Leukotrienes Med; 8(3):191-198 1982

Nitrosomethylurea-induced mammary tumors of Buffalo rats were used to study the regression after oophorectomy (oox). The amounts of prostaglandin E2 (PGE2) and prostaglandin F2 alpha (PGF2a) isolated from tumor cells membranes increased during the period of tumor growth, peaking 5 days after oophorectomy. The membrane binding of both prostaglandins (PGs) was saturable and reversible, reaching equilibrium after 30 min. The membrane binding of both PGs was 3-4x above baseline values on day 4 after oox. The membrane binding of PGE2 showed low-affinity characteristics during both tumor growth and oox-induced tumor regression. The membrane binding of PGF2a showed high-affinity characteristics during both tumor growth and oox-induced tumor regression; the post-oox increase in specific PGF2a binding was attributed to an increase in the number of PGF2a receptors. The elevated rates of PGF2a synthesis and the regression-associated increase in PGF2a receptors suggest that PGF2a may play a significant role in hormone-dependent mammary tumor regression. (22 Refs)

48. PROSTAGLANDIN D2, A POTENTIAL ANTINEOPLASTIC AGENT.

Fukushima M, Kato T, Ueda R, Ota K, Narumiya S, Hayaishi O
Dept. Internal Medicine, Aichi Cancer Center, Chikusa-ku, Nagoya 464, Japan
Biochem Biophys Res Commun; 105(3):956-964 1982

Evidence is presented that prostaglandin D2 (PGD2) has a potent cytotoxic activity against several lines of tumor cells such as L1210 mouse leukemia cells and several human leukemia cells in vitro. The concentration needed for 50% growth inhibition (IC50) of L1210 cells was 2.4 ug/ml, approximately equivalent to the IC50 of bleomycin under similar experimental conditions. Similar cytotoxic action of PGD2 against cultured mastocytoma cells has been recently reported. Similar growth

inhibition was observed at similar PGD2 concentrations for several human leukemia cell lines, including NALL-1, RPMI-8226, RPMI-8402, and SK-LY-16. Results of preliminary experiments indicate that PGD2 injected daily ip (0.5 mg/kg) decreases the growth of Ehrlich tumor inoculated sc in the axillar regions of ICR mice. Of other prostaglandins tested, only prostaglandin E2 and prostaglandin A2 showed significant growth inhibition. Prostaglandin B2, prostaglandin F2alpha, and prostaglandin I2 had no effect on cell proliferation even at relatively high concentration (14.3 uM). (17 Refs)

49. CELL GROWTH AND THE REGULATION OF PROSTAGLANDIN SYNTHESIS.

Taylor L, Polgar P
Dept. Biochemistry, Boston Univ. Sch. Medicine, Houseman Building, Boston, MA, 02118
Prostaglandins; 22(5):723-728 1982

Prostaglandin (PG) synthesis was determined in human embryo lung fibroblasts (HELF) during active, slowed and non-growing phases. Bradykinin and ascorbic acid were used to induce PG synthesis. The cells were also exposed to arachidonic acid, a PG precursor. During active growth, PGE2 synthesis in response to stimulation by either bradykinin or ascorbic acid was low. As growth slowed the cellular response changed. During quiescence bradykinin and ascorbic acid stimulated PG production markedly while the conversion of free arachidonic acid to PGE2 also increased markedly. This change in response by quiescent cells was not due to an increase in cell density. When growing and quiescent cells at the same cell density were compared, the growing cells showed very little response to bradykinin while the quiescent cells were very responsive. The change in response was also not due to any differences in arachidonic acid concentrations in the culture medium during growth and non-growth. (Author abstract) (11 Refs)

50. CHANGES IN PROSTAGLANDIN LEVELS IN CULTURES OF SV40-TRANSFORMED MACROPHAGE CELL LINES IN RELATION TO THEIR PHENOTYPIC EXPRESSION.

Tanigawa T, Suzuki T, Takayama H, Takagi A
Dept. Bacteriology, Tottori Univ. Sch. Medicine, Yonago, Tottori 683, Japan
Microbiol Immunol; 26(1):59-66 1982

Prostaglandin (PRG) levels were determined in culture fluids of simian virus 40-transformed cells, line 28-12 and its subline, and the release of PRG by the cells when they are treated with lipopolysaccharide (LPS) and OK-432 (a streptococcal preparation from penicillin-attenuated Streptococcus pyogenes) was investigated. Low levels of PRG were detected in Dulbecco's modified Eagle's medium, and significantly higher levels (4,921 +-140 picog/ml) were detected in heat-inactivated fetal calf serum. 28-12 cells were cultivated for 16 days and the medium was changed every 4 days. A low level of PRG was detected in 4-day culture medium during the growing phase of the cells. When the proportion of phagocytic cells increased during the stationary phase, a decrease in PRG levels was observed. The levels of PRG remained lower than background levels and constant during the culture period when the 28-12 subline isolated by cultivation with cytosine arabinoside (28-12Ara) was cultivated without a change in the medium for 4 days. Non-phagocytic 28-12 cells and phagocytic 28-12Ara cells were cultivated for 24 hr in the presence and absence of LPS (1 ug/ml) and OK-432 (25 ug/ml). The release of PRG by 28-12Ara cells was markedly enhanced, whereas its release by 28-12 cells was not affected. The results indicate the correlation between the phenotypic change from the nonphagocytic to the phagocytic state and a decrease in PRG levels in culture fluids, and that phagocytic cells are responsive to PRG inducers, whereas nonphagocytic cells are not. (27 Refs)

51. PGE1 AND PGE2 PATTERNS IN NORMAL AND LEUKEMIC GRANULOCYTES DURING IN VITRO PHAGOCYTOSIS.

Notario A, Azzara A, Petrucci E, Invernizzi R, Luraschi A
Istituto di Clinica Medica Generale e Terapia Medica I, Universita di Pavia, Pavia, Italy
Arch Sci Med (Torino); 139(1):1-8 1982

Behaviors of prostaglandins E1 and E2 (PGE1 and PGE2) during in vitro phagocytosis (PC) of latex particles by granulocytes of normal controls (NC: 10) and leukemic patients (22) were studied with the aid of an immunoenzymatic method using peroxidase-antiperoxidase. The patients consisted of five with chronic myeloid leukemia (CML), five with acute myeloblastic leukemia (AML), five with chronic lymphatic leukemia (CLL), four with acute lymphoblastic leukemia (ALL), and three with acute myelomonocytic leukemia (AMML). The PC was graded from 0 (when there was none) to +++ (when it was complete). The Grade +++ PC was achieved by 80 +-7% of the NC, 83 +-15% of the CLL, 76 +-9% of the ALL, 41 +-18% of the AML, 13 +-5% of the AML, and 6 +-5% of the AMML. The Grade 0 PC was less than 10% in the NC, ALL, CLL, and AMML, but 40% in the CML and 62% in the AML. The PC of the AMML was less than 10% in all grades except Grade ++, where it was 28%. The levels of PGE1 and PGE2 in the CML were close to those in the NC, were lowest in the AML, and were next to lowest in AMML. The PGE1 and PGE2 (especially the PGE2) were active during the PC, as was demonstrated by their redistribution into the cells that were engulfing foreign particles and by their accumulation along the cell membranes and the engulfed foreign particles. (28 Refs) (In ITA)

52. ROLE OF PHOSPHOLIPASE A2 AND PROSTAGLANDIN E IN GROWTH AND DIFFERENTIATION OF MYELOID LEUKEMIC CELLS.

Simantov R, Sachs L
Dept. Genetics, Weizmann Inst. Science, Rehovot 76100, Israel
Biochim Biophys Acta; 720(2):111-119 1982

Phospholipase A2 activity and prostaglandin E synthesis have been studied in different clones of myeloid leukemic cells, which differ in their competence to be induced to differentiate by the macrophage and granulocyte differentiation-inducing protein or the tumor promoter 12-O-tetradecanoyl phorbol-13-acetate (TPA). Clones that could be induced to differentiate by this protein showed a higher basal phospholipase A2 activity than clones that could not be induced to differentiate by this protein inducer. Cell competence to be induced to differentiate by TPA did not show this correlation, and the clone with the least ability to respond to TPA showed the lowest number of binding sites for [20-3H]phorbol 12,13-dibutyrate. Differentiation induced by the protein was accompanied by a 7-14-fold increase in prostaglandin E synthesis, whereas differentiation induced by TPA did not show this increase. Externally added prostaglandin E1 did not induce differentiation but inhibited cell proliferation and the degree of inhibition in the different clones was related to the basal phospholipase A2 activity. The results indicate that increase of prostaglandin E synthesis was not an essential pre-requisite for differentiation, that prostaglan-

din E seems to be involved in the inhibition of cell proliferation in association with phospholipase A2, and that the differentiation-inducing protein and TPA can induced differentiation by different pathways. The amount of basal phospholipase A2 activity was also related to previously found differences in the ability of the clones to develop desensitization to beta-adrenergic hormones or prostaglandin E1. (Author abstract) (25 Refs)

CYCLIC NUCLEOTIDE/ POLYAMINES/ NUCLEOTIDES AS GROWTH EFFECTORS

53. MICROSOMAL CAMP-INDEPENDENT HISTONE H1 KINASE ACTIVITY IN PLASMACYTOMA, MORRIS HEPATOMA AND NORMAL LIVER.

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Biochimie; 64(1):13-20 1982

A protein kinase activity with high specificity for histone H1 was isolated from mouse plasmacytoma, Morris hepatoma and normal mouse liver and compared by ion exchange chromatography after DEAE-cellulose, hydroxylapatite and Sephadex G-200 chromatography. This cAMP-independent histone H1 kinase is not affected by the heat-stable cAMP-dependent protein kinase inhibitor. It has the following particular properties: it prefers GTP to ATP as substrate and was found to be present with a great activity only in neoplastic tissues. No phosphatase activity was detected in the partially purified histone H1 kinase fraction from normal and neoplastic cells. These results suggest either an increased amount of histone H1 kinase and/or of its activator in neoplastic cells, or the presence of a strong inhibitor in normal cells. This histone H1 kinase appears to be analogous to the chromatin bound kinase which phosphorylates histone H1 at the NH2 and COOH terminal regions. We might suggest an implication of this kinase in the regulation of cell division. (Author abstract) (12 Refs)

54. CAMP DIFFERENCES BETWEEN CLONES OF HIGH AND LOW MALIGNANT FIBROSARCOMA CELLS.

Elgebaly S, Kunkel S, Lovett EJ, Varani J
Dept. Pathology, Univ. Connecticut Health Center, Farmington, CT
Oncology; 39(3):163-166 1982

The levels of 3',5'-cyclic adenosine monophosphate (cAMP) were measured in a high malignant and a low malignant clone of murine fibrosarcoma cells isolated from a common parent and in normal mouse fibroblasts at various stages of growth. There was an inverse correlation between the degree of malignant potential and the cAMP levels. The normal fibroblasts had the highest cAMP at all time periods measured. The high malignant cells had the lowest levels and the low malignant cells were intermediary. The degree of susceptibility of each population to the growth inhibiting effects of dibutyryl cAMP, a cAMP analogue, was also measured. There was a direct correlation between degree of malignant potential and susceptibility to growth inhibition. These findings strongly support our belief that the low malignant cells represent a state of malignancy that is intermediary between the high malignant cells and the normal fibroblasts. cAMP differences may contribute to this. (Author abstract) (14 Refs)

55. CHANGES IN CYCLIC AMP-DEPENDENT PROTEIN KINASES DURING INHIBITION OF MASTOCYTOMA CELL GROWTH BY DIBUTYRYL CYCLIC AMP.

Evans J, Smart J, Airey P, Ralph R
Dept. Cell Biology, Univ. Auckland, Auckland, New Zealand
Mol Cell Biochem; 43(3):183-190 1982

Inhibition of growth of PY815 mouse mastocytoma cells in vitro by N6,O2'-dibutyryladenosine 3',5' cyclic monophosphate (DB cyclic AMP) was accompanied by increases in intracellular cyclic AMP and histamine and minor changes in cytosolic cyclic AMP-dependent histone kinase activity. However, DEAE-cellulose chromatography revealed substantial changes in the relative proportions of the principal cyclic AMP-dependent protein kinases and in free cyclic AMP-binding protein after DB cyclic AMP treatment. The activity of cytosolic cyclic AMP-dependent protein kinase Type I (PKI) decreased relative to cyclic AMP-dependent protein kinase Type II (PKII) and there was an increase in a cytosol cyclic AMP-binding protein with little associated protein kinase activity. The relative changes in activity of PKI, PKII and cyclic AMP binding protein after DB cyclic AMP treatment may reflect events important in the regulation of growth and differentiation of mast cells. (Author abstract) (43 Refs)

COFACTORS/ NUTRITIONAL GROWTH FACTORS

56. ISOLATION OF METHYLCHOLANTHRENE-'INITIATED' C3H/10T1/2 CELLS BY INHIBITING NEOPLASTIC PROGRESSION WITH RETINYL ACETATE.

Mordan LJ, Bergin LM, Budnick JE, Meegan RR, Bertram JS
Dept. Experimental Therapeutics, Roswell Park Memorial Inst., 666 Elm St., Buffalo, NY, 14263
Carcinogenesis; 3(3):279-285 1982

A clone of 3-methylcholanthrene-treated 10T1/2 cells has been isolated which possess basic characteristics expected of 'initiated' cells. In the presence of retinyl acetate, this clone exhibits contact inhibited growth control and is morphologically indistinguishable from the parental 10T1/2 cell line. Removal of retinyl acetate in vitro results in neoplastic transformation after a latent period of 3 wk. The classical 10T1/2 transformation system was reconstructed by coculturing normal and 'initiated' 10T1/2 cells. In these cultures 62% of the clonogenic 'initiated' 10T1/2 cells formed either Type III foci after a latent period of 3-4 wk, and an additional 22% formed Type I foci. Treatment of 'initiated' 10T1/2 cells with the tumor promoter 12-O-tetradecanoylphorbol-13-acetate accelerated the formation of transformed foci in the coculture system by reducing the length of the latent period to less than 3 wk. Injection of 10(6) 'initiated' cells/mouse sc into nude mice resulted in the appearance of progressively growing fibrosarcomas after a latent period of 5-7 wk. Dietary supplementation with 4-hydroxyphenyl-retinamide prevented tumor formation; after drug withdrawal, tumors developed in all surviving mice after 6 wk. We believe this cell line possess all characteristics expected of 'initiated' cells. With this new cell line, designated INIT/10T1/2, we can now study the early biochemical changes in growth control mechanisms resulting in neoplastic transformation and the mechanism(s) of chemoprevention of cancer by vitamin A. (Author abstract) (31 Refs)

57. RETINOIC ACID-PROMOTED EXPANSION OF TOTAL CELLULAR ATP POOLS IN 3T3 CELLS CAN MEDIATE ITS STIMULATORY AND GROWTH INHIBITORY EFFECTS.

Rapaport E, Schroder EW, Black PH
Dept. Microbiology, Boston Univ. Sch. Medicine, Boston, MA, 02118
J Cell Physiol; 110(3):318-322 1982

Levels of beta-all-trans-retinoic acid (RA) which stimulate the phorbol myristate acetate (PMA)-induced transition from resting to growing states in 3T3 cells following short periods of treatment were found to produce marked increases in total cellular ATP and uridine triphosphate pools of the resting cells. Total cellular ATP pools increased by about twofold during transition of cells from G1 phase to S phase of their cell cycle. Treatment of serum-deprived cultures with 10 uM RA for at least 48 hr prior to serum stimulation and up to the time of analysis increased total cellular ATP pools during all phases of the cell cycle. Treatment of asynchronous cells with 10 uM RA for 72 hr produced no difference in labeling indices compared to controls, despite a 40% inhibition of (3H)thymidine incorporation. Exposure of G0/G1-arrested 3T3 cells to 10 uM RA for 18 hr followed by removal of the RA-containing medium and addition of 50 nanogram/ml PMA resulted in a substantial potentiation of the mitogenic response to PMA. These results indicated that RA treatment of 3T3 cells lengthened the S phase of their cycle by reducing the rate of DNA synthesis. (21 Refs)

58. EFFECTS OF RETINOIC ACID ON THE BINDING AND MITOGENIC ACTIVITY OF EPIDERMAL GROWTH FACTOR.

Jetten AM
Lab. Experimental Pathology, Div. Cancer Cause and Prevention, NCI, Bethesda, MD, 20205
J Cell Physiol; 110(3):235-240 1982

The effects of retinoids upon internalization and degradation of epidermal growth factor and on the mitogenic stimulation by EGF in BALB/c 3T6 mouse fibroblasts grown in culture were examined. Levels of total cell-associated binding of EGF in retinoic acid-treated cells was enhanced several fold over controls. In both retinoic acid-treated and control cells the majority of cell-associated EGF binding appeared to be internalized. Both total cell-associated and internalized 125I-EGF were sharply reduced after 2-3 hr of incubation, probably due to the degradation of the growth factor in the lysosomes and subsequent secretion of 125I-labelled tyrosine. Even in the absence of internalization and degradation, retinoic acid treatment enhanced binding of EGF to 3T6 cells. Retinoic acid treatment enhanced the available EGF receptor sites from approx 16,000 receptors per cell for control cells to approx 140,000 receptors per cell for retinoic acid-treated cells. No dramatic differences were observed in rates of EGF internalization and degradation following retinoic acid treatment. The rate at which EGF binding is restored after down-regulation is about three times as fast as in control cells. No direct antagonism of EGF binding was observed between the tumor promoter 12-O-tetradecanoyl-phorbol-13-acetate and retinoic acid. (28 Refs)

59. RETINOID-BINDING PROTEINS.

Goodman DS
Div. Metabolism and Nutrition, Dept. Medicine, Columbia Univ. Coll. Physicians and Surgeons, New York, NY, 10032
J Am Acad Dermatol; 6(4,part2):583-590 1982

Plasma retinol binding protein (PRBP), cellular retinol binding protein (CRBP), and cellular retinoic acid binding protein (CRABP) are immunologically distinct from each other. PRBP, produced in the liver and catabolized mainly in the kidneys, is responsible for the delivery of retinol to its extrahepatic sites of action. No disease has yet been identified in which PRBP was totally absent or immunologically abnormal. The results of preliminary experiments suggest that CRBP may be identical in different tissues and different species. Comparable information is not yet available for CRABP. The tissue levels and distributions of CRBP and CRABP in animal and human tissues are distinct from each other. Little is known about the factors influencing and regulating the tissue levels and metabolism of CRBP and CRABP. Retinoid-binding proteins (notably CRABP) have been identified in many tumors and cell lines. It has been reported that the binding ability of synthetic retinoids for the intracellular binding proteins (notably CRABP) tends to correlate with the ability of the retinoid to influence epithelial cell differentiation and/or tumor growth in vivo or in vitro. Further research is needed to define the biochemical processes mediated by these substances. (57 Refs)

60. RETINOIC ACID-INDUCED GROWTH INHIBITION AND MORPHOLOGIC DIFFERENTIATION OF HUMAN NEUROBLASTOMA CELLS IN VITRO.

Sidell N
Div. Surgical Oncology, Univ. California Los Angeles Sch. Medicine, Univ. California, Los Angeles, CA, 90024
JNCI; 68(4):589-596 1982

Retinoic acid (RA) induced concentration-dependent morphologic differentiation and growth inhibition in the LA-N-1 human neuroblastoma cell line. Time course studies demonstrated a significant increase in the formation of long neurites in LA-N-1 cultures within 48 hr of RA addition; maximum expression of differentiation occurred at approx 4 days. This differentiation profile corresponded to a detectable decrease in [3H]thymidine incorporation at 48 hr and complete inhibition of cell growth after 3-4 days. The RA-induced morphologic differentiation and growth inhibition persisted despite removal of the drug. A soft agar assay system showed that RA also inhibited the ability of LA-N-1 cells to form anchorage-independent colonies and induced morphologic differentiation in colonies that did develop. These findings suggest that RA promoted the differentiation of LA-N-1 neuroblastoma cells, resulting in an altered expression of the malignant phenotype. (Author abstract) (22 Refs)

61. RETINOIC ACID ENHANCES GROWTH OF HUMAN EARLY ERYTHROID PROGENITOR CELLS IN VITRO.

Douer D, Koeffler HP
Dept. Medicine Div. Hematology/Oncology, Univ. California Los Angeles Sch. Medicine, Los Angeles, CA, 90024
J Clin Invest; 69(4):1039-1041 1982

We studied the effect of retinoic acid on the clonal proliferation of normal human early erythroid progenitor cells in vitro. Normal peripheral blood cells were cultured in methylcellulose with erythropoietin and the number of burst-forming units-erythroid (BFU-E) colonies were scored on Day 12 of culture. All-trans retinoic acid increased the number of colonies in a dose-response fashion. Maximal stimulation occurred at 30 nM retinoic acid, which increased the number of BFU-E by a mean of 225 +-25% (+-SE) over plates containing erythropoietin alone. Colony formation increased even in the presence of maximally stimulating concentrations of erythropoietin. The 13-cis retinoic acid stimulated BFU-E proliferation in a parallel manner as the trans analogue, while retinol (vitamin A) did not

affect clonal growth. This data supports further the thesis that retinoic acid, in addition to its known effect on epithelial cells, may be involved in the growth of normal hematopoietic cells. (Author abstract) (12 Refs)

62. RETINOIDS AND INHIBITION OF ORNITHINE DECARBOXYLASE ACTIVITY.

Boutwell RK, Lowe NJ, DeLuca LM, Meyskens FL, McGuire JS, Krueger GG, Goldsmith LA, Kligman AM
McCardle Lab. for Cancer Res., Univ. Wisconsin, Madison, WI, 53706
J Am Acad Dermatol; 6(4,part2):796-800 1982

A model system for production of skin tumors involves a two stage system in which the skin is first exposed to a low dose of a carcinogen (initiator) which has an irreversible effect on epidermal stem cells. The second step involves exposure of skin to agents, which although not carcinogenic, will cause many tumors quickly (promoter). The second agent causes increased somatic cell gene activity, in this case, induction of ornithine decarboxylase (ODC) activity. The tumor promoter, 12-O-tetradecanoylphorbol-13-acetate (TPA) caused a dose dependent induction of ODC activity in mouse skin within 5 hr of a skin application of 1-10 nanomoles. Induction was inhibited by a single application of from 0.034-3.4 nanomoles of retinoic acid (RA) immediately before or after TPA treatment. Of 30 retinoids tested, all trans-RA was most effective. The amount of inhibition of ODC induction was dependent on time of application of RA relative to time of TPA application, and was also correlated with dose and RA structure. A permanently increased level of ODC and its product, putrescine, may be an important component of tumor promotion. (18 Refs)

63. THE EFFECT OF 1,25(OH)2D3 ON ALKALINE PHOSPHATASE IN OSTEOBLASTIC OSTEOSARCOMA CELLS.

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J Biol Chem; 257(7):3362-3365 1982

The effects of 1,25-dihydroxyvitamin D3 on growth, cell protein, and alkaline phosphatase were examined in cultures of a clonal osteoblast-like rat osteosarcoma cell line at different stages of phenotypic maturation, defined by the level of alkaline phosphatase activity. In 'immature' cultures with low alkaline phosphatase specific activity, 1,25-dihydroxyvitamin D3 at 10(-8) -10(-7) M inhibited cell growth and elevated alkaline phosphatase activity; lower hormone concentrations had no effect. In cultures with higher alkaline phosphatase activity, the hormone had biphasic effects: at concentrations of 10(-8) - 10(-7) M, 1,25-dihydroxyvitamin D3 inhibited growth and elevated alkaline phosphatase and total cell protein; however, lower hormone concentrations (10(-10) and 10(-9) M) reduced alkaline phosphatase activity. This effect was more pronounced in more 'mature' cultures where half-maximum inhibition of alkaline phosphatase activity occurred near 10(-11) M. At this stage, the hormone caused a small increase in cell number. The data suggest that 1,25-dihydroxyvitamin D3 exerts differential effects on skeletal cells depending on their state of growth or maturation. This finding may offer new insights into the effects of 1,25-dihydroxyvitamin D3 on bone and may help resolve apparently conflicting experimental findings on the effects of the hormone on osteoblast-like cells in culture. (Author abstract) (27 Refs)

64. EFFECT OF DL-ALPHA-TOCOPHEROL (VITAMIN E) ON THE DIFFERENTIATION ON MOUSE MYELOID LEUKEMIA CELL.

Sakagami H, Asaka K, Abe E, Miyaura C, Suda T, Konno K
Dept. Biochemistry, Sch. Medicine, Showa Univ., 1-5-8 Hatanodai, Shinagawa-ku, Tokyo 142, Japan
J Nutr Sci Vitaminol; 27(4):291-300 1981

Mouse M1 (clone T22) myeloid leukemia cells in suspension cultures were exposed to DL-alpha-tocopherol (AT: up to 80 uM). When AT was solubilized into the culture medium, it was relatively stable (half-life, 147-168 hr). The uptake of AT into the M1 cells was time- and dose-dependent. High concentrations of AT slightly enhanced M1 cell proliferation; no concentration at AT inhibited cell proliferation. Physiologic concentrations of AT (2-80 uM) slightly inhibited both spontaneous and dexamethasone-induced cell differentiation (as assessed by adhesion characteristics and surface rosette formation), but the AT-exposed cells did not revert to the baseline percentage of undifferentiated cells. AT exposure also caused a 2-fold increase in acid phosphatase activity; the relationship between this phenomenon and cell differentiation is being studied. The AT-exposed cells became rounder in shape, perhaps as a consequence of surface membrane changes after the hydrophobic interaction of AT with some membrane components. (19 Refs)

EXOGENOUS AGENTS AFFECTING DIFFERENTIATION

65. THE RELATIONSHIP BETWEEN DIFFERENTIATION OF FRIEND ERYTHROLEUKEMIA CELLS AND SYNTHESIS OF PLASMA MEMBRANE-BOUND GLYCOPROTEINS.

Bosman GJ, Boer P, Steyn-Parve EP
Lab. for Physiological Chemistry, State Univ. Utrecht, Vondellaan 24A, 3521 GG Utrecht, The Netherlands
Protides Biol Fluid Proc Colloq; 29:67-70 1982

Changes that plasma membrane-bound glycoproteins go through during dimethyl sulfoxide (DMSO)-induced differentiation of Friend virus-transformed murine erythroleukemia cells (FELC) are reported. When FELC were induced with DMSO in the presence of tunicamycin, Hb synthesis was inhibited, seemingly by arresting the cells just before they became irreversibly committed to differentiation. Results suggest that changes in synthesis of plasma membrane-bound glycoproteins may play a role in the differentiation of Friend erythroleukemia cells. (11 Refs)

66. INVOLVEMENT OF POLY(ADP-RIBOSE) METABOLISM IN INDUCTION OF DIFFERENTIATION OF HL-60 PROMYELOCYTIC LEUKEMIA CELLS.

Kanai M, Miwa M, Kondo T, Tanaka Y, Nakayasu M, Sugimura T
Natl. Cancer Center Res. Inst., Tsukiji, Chuo-ku, Tokyo 104, Japan
Biochem Biophys Res Commun; 105(2):404-411 1982

The metabolism of poly(ADP-ribose) during the dimethylsulfoxide (DMSO)-induced differentiation of cultured human promyelocytic leukemia HL-60 cells was measured at 14 hr, 3 days, 5 days, and 7 days following DMSO treatment. There were no phenotypic changes within 14 hr after treatment. By day 3, 45% of the cells exhibited differentiated morphology of myelocytes, metamyelocytes and mature granulocytes; by day

5-7 about 80% of the cells had differentiated. By day 7, 70% of the cells phagocytized latex particles compared to 2% of the control cells. Myeloperoxidase activity decreased to 78% of the uninduced level after 14 hr, and to 24% after 3 days, remaining at this level through day 7. Following DMSO treatment, the level of endogenous poly(ADP-ribose) decreased to 15% of the uninduced level for 14 hr, remained near this level until day 3, then increased to 800% of the uninduced level by day 5. The poly(ADP-ribose) polymerase activity decreased to 40% of the uninduced level after 14 hr and remained at this level for 7 days. The poly(ADP-ribose) hydrolyzing activity in treated cells was the same as the controls. Thus, endogenous poly(ADP-ribose) rapidly decreases during the early stages of differentiation and then increases greatly with the expression of differentiated phenotypes. These changes apparently cannot be explained by changes in poly(ADP-ribose) polymerase and hydrolase activity. (31 Refs)

67. INHIBITORS OF PROTEIN GLYCOSYLATION INHIBIT THE DIFFERENTIATION OF FRIEND ERYTHROLEUKEMIA CELLS.

Bosman GJ, Boer P, Steyn-Parve EP
Lab. Physiological Chemistry, State Univ. Utrecht, Vondellaan 24a, 3521 GG Utrecht, The Netherlands
Biochim Biophys Acta; 696(3):285-289 1982

Tunicamycin, 2-deoxy-D-glucose and 2-deoxy-2-fluro-D-glucose inhibit dimethyl sulfoxide-induced differentiation of Friend cells. This inhibition, characterized by inhibition of hemoglobin synthesis, is accompanied by a specific inhibition of protein glycosylation. The results of cloning experiments indicate that this inhibition specifically affects cells in the period preceding their commitment. These results suggest that glycoprotein synthesis is a requirement for Friend erythroleukemia cells in order to initiate the expression of the terminal differentiation program. (Author abstract) (13 Refs)

68. LOSS OF TRANSFERRIN RECEPTORS FOLLOWING INDUCED DIFFERENTIATION OF HL-60 PROMYELOCYTIC LEUKEMIA CELLS.

Yeh CJ, Papamichael M, Faulk WP
Blond McIndoe Centre for Transplantation Biology, Queen Victoria Hosp., East Grinstead, Sussex RH19 3DZ, England
Exp Cell Res; 138(2):429-433 1982

Human promyelocytic leukemia (HL-60) and lymphoblastoid (Daudi) cells were studied for transferrin receptors before and after induced differentiation with dimethyl sulfoxide (DMSO), sodium butyrate or retinoic acid. None of these reagents affected the morphology or presentation of receptors in Daudi cells, but many HL-60 morphologically matured to banded neutrophils and demonstrated a concomitant loss of transferrin binding, suggesting an important role of transferrin receptors in cellular differentiation. (Author abstract) (31 Refs)

69. ENHANCEMENT OF HORMONE ACTION BY A PHORBOL ESTER AND ANTI-TUBULIN ALKALOIDS INVOLVES DIFFERENT MECHANISMS.

Simantov R, Sachs L
Dept. Genetics, Weizmann Inst. Science, Rehovot 76100, Israel
Biochim Biophys Acta; 720(2):120-125 1982

The tumor-promoting phorbol ester 12-O-tetradecanoyl phorbol-13-acetate (TPA) enhanced 1-isoproterenol and prostaglandin E1 stimulated cyclic AMP formation in clones of mouse myeloid leukemic cells. The enhancement was found up to 3 hr after TPA treatment and had disappeared after 24 hr, indicating its reversibility. The effect of TPA was not inhibited by removal of extracellular Ca++ or pretreatment with the calcium ionophore A23187. This enhancement by TPA seems to involve a different pathway than enhancement of response to the same hormones after treatment with the anti-tubulin alkaloids colchicine or vinblastine, since a myeloid leukemic cell mutant clone that was non-responsive to the anti-tubulin alkaloids responded to TPA. Furthermore, combined treatment of colchicine-sensitive cells with TPA and colchicine showed an additive stimulating effect. The enhancement of cell response to hormones by TPA was found in myeloid leukemic cell clones that either were or were not induced to differentiate after treatment with TPA. This suggests that enhancement of the effect of these and possibly other hormones by TPA may be an initial step of TPA action, but that this enhancement is not sufficient to induce the wide repertoire of TPA effects including induction of differentiation. (Author abstract) (36 Refs)

70. DIFFERENTIAL EFFECT OF DEXAMETHASONE ON FRIEND LEUKEMIA CELLS INDUCED TO DIFFERENTIATE BY HEXAMETHYLENEBISACETAMIDE OR BUTYRIC ACID.

Gambari R
Inst. di Biologia Generale, Univ. di Roma, Rome, Italy
Cell Biol Int Rep; 6(4):341 1982

The effects of dexamethasone (DEX) on erythroid function in murine Friend leukemia (FL) cells, clone DS19-4, induced to differentiate by hexamethylenebisacetamide (HMBA) or butyric acid (BA) were investigated. Synthesis and translation of alpha- and beta-globin mRNA and Hb accumulation were greatly inhibited by DEX in the HMBA-induced cells. DEX had no effect on these functions in the BA-induced cells. The results suggest that HMBA and BA modulate gene expression in FL cells via a different mechanisms. The DS19-4 cells cultured with BA may share cellular properties in common with FL clones resistant to DEX inhibition of HMBA-induced erythroid differentiation. (5 Refs)

71. INDUCTION OF DIFFERENTIATION IN HUMAN PROMYELOCYTIC LEUKEMIA CELLS BY TUMOR PROMOTERS.

Nakayasu M, Terada M, Adolf W, Opferkuch HJ, Schmidt R, Hecker E, Sugimura T
Div. Biochemistry, Natl. Cancer Center Res. Inst., Tsukiji, Chuo-ku, Tokyo 104, Japan
J Cancer Res Clin Oncol; 103(1):17-29 1982

The prototype polyfunctional diterpene ester tumor promoter of two-step carcinogenesis in mouse skin, 12-O-tetradecanoylphorbol-13-acetate (TPA), induced differentiation of human promyelocytic leukemia cells (HL-60) in culture. Differentiation of HL-60 cells was characterized by increased phagocytosis, increased lysozyme activity (EC 3.2.1.17) in the growth medium, and changes in morphology to those characteristics of more mature cells resembling macrophages. Many of the cells treated with TPA became aggregated, attaching firmly to culture flasks. The av intracellular myeloperoxidase activity (EC 1.11.1.7) per cell decreased during induction of differentiation by TPA. It was also found that TPA enhanced, rather than inhibited, differentiation of HL-60 cells induced by DMSO. In addition to TPA, several polyfunctional diterpene esters of the tigliane, ingenane, and daphnane type have been tested for their ability to induce morphological and functional changes of HL-60 cells. The activities of the compounds to

induce these changes correlated well with their activities as tumor promoters in two-step carcinogenesis in mouse skin. In particular, half the concentrations required for induction of adhesion of the cells to flasks were roughly correlated to the potency of these compounds as tumor promoters. Among the compounds tested, phorbol-12,13-didecanoate (PDD), ingenol-3-hexadecanoate, Pimelea factor P1 and Pimelea factor P2 were as active as TPA, while methyl-TPA and 4alpha-PDD were much less active. Phorbol and ingenol were totally inactive up to a concentration 10,000-fold higher than that of TPA. (Author abstract) (28 Refs)

72. DIVERGENT RESPONSES IN EPIDERMAL BASAL CELLS EXPOSED TO THE TUMOR PROMOTER 12-O-TETRADECANOYLPHORBOL-13-ACETATE.

Yuspa SH, Ben T, Hennings H, Lichti U
In Vitro Pathogenesis Section, NCI, Bethesda, MD, 20205
Cancer Res; 42(6):2344-2349 1982

Mouse epidermal basal cells can be selectively cultivated in medium with 0.02 to 0.09 mM Ca++ and can be induced to differentiate by medium containing 1.2 mM Ca++. Basal cell cultures were studied to determine if all cells in this population responded identically to the skin tumor promoter 12-O-tetradecanoylphorbol-13-acetate (TPA). Studies on the induction of the enzyme epidermal transglutaminase by TPA demonstrated a two- to four-fold increase in activity within 12 hr of exposure. This activity increase paralleled morphological differentiation in approx 50% of the basal cell population, and differentiating cells sloughed from the culture dish within 24 to 48 hr as transglutaminase activity returned to basal levels. The cells which remained were resistant to induced differentiation by 1.2 mM Ca++ medium, in that they failed to demonstrate increased transglutaminase activity or decreased thymidine incorporation, both characteristics of control basal cells induced to differentiate by 1.2 mM Ca++. Cells remaining after a single exposure to TPA did not respond to a second exposure with an induction of transglutaminase if the interval between exposures was 4 days. TPA-pretreated cells did not undergo a transient decrease in thymidine incorporation (characteristic of control cells) when exposed to TPA a second time but instead were directly stimulated to proliferate by the phorbol ester, indicating that such cells were not refractory to the promoter. When the treatment-free interval after TPA was extended from 4 to 10 days, transglutaminase inducibility was restored in basal cells to either TPA or 1.2 mM Ca++ as inducers. These results indicate that heterogeneity exists within the epidermal cell population and that exposure to phorbol esters induces differentiation in some cells, while stimulating proliferation in others. Such heterogeneous responses would cause a selective redistribution of the epidermal cell population and could lead to clonal expansion of initiated cells. (Author abstract) (34 Refs)

73. MODULATION OF CHRONIC LYMPHOCYTIC LEUKEMIA CELLS BY PHORBOL ESTER: INCREASE IN IA EXPRESSION, IGM SECRETION AND MLR STIMULATORY CAPACITY.

Okamura J, Letarte M, Stein LD, Sigal NH, Gelfand EW
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J Immunol; 128(5):2276-2280 1982

When cultured with 12-O-tetradecanoylphorbol 13-acetate (TPA) at a concentration of 1.6 x 10(-7) M, chronic lymphocytic leukemia (CLL) cells differentiated into mature cells of B lineage and increased their expression of surface Ia antigens when compared with cells cultured in the absence of TPA. Concurrently, TPA enhanced the ability of CLL cells to stimulate in a mixed lymphocyte reaction (MLR). The events induced in vitro by TPA that are characteristic of B cell maturation included morphologic changes, reduction in surface immunoglobulin (Ig), appearance of cytoplasmic Ig, and secretion of IgM. The increase in Ia expression and the enhanced capacity to stimulate in an MLR after incubation with TPA might also be associated with maturation of the CLL cells. The changes induced in vitro by TPA in neoplastic B cells provide new information concerning the terminal events in normal B cell differentiation. (Author abstract) (24 Refs)

74. POLYAMINE BIOSYNTHESIS AND SKIN TUMOR PROMOTION: INHIBITION OF 12-O-TETRADECANOYLPHORBOL-13-ACETATE-PROMOTED MOUSE SKIN TUMOR FORMATION BY THE IRREVERSIBLE INHIBITOR OF ORNITHINE DECARBOXYLASE ALPHA-DIFLUOROMETHYLORNITHINE.

Takigawa M, Verma AK, Simsiman RC, Boutwell RK
McArdle Lab. Cancer Res., Univ. Wisconsin, Madison, WI, 53706
Biochem Biophys Res Commun; 105(3):969-976 1982

Topical application of alpha-difluoromethylornithine (DFMO) to shaved mouse skin or administered in the drinking water in conjunction with the tumor promoter 12-O-tetradecanoylphorbol-13-acetate (TPA), inhibited the formation of 7,12-dimethylbenz(a)anthracene-initiated skin papillomas. Application of 10 nanomoles of TPA to mouse skin led to a rapid and transient induction of epidermal ornithine decarboxylase (ODC) activity. Application of 1.37 umoles DFMO 1 hr after TPA to mouse skin inhibited ODC induction by nearly 100%. DFMO also resulted in significant inhibition of induced and basal levels of putrescine, while spermidine-induction was inhibited by only 20%. DFMO administered in the drinking water (1% wt/vol) beginning 3 days before and continuing through the experimental period inhibited TPA-induced increases in ODC by 100%, basal putrescine levels by 40%, and induced putrescine levels by 90%; spermidine levels were somewhat decreased, but spermine levels remained unchanged. DFMO inhibited the formation of DMBA-initiated and TPA-promoted skin papillomas by 50% when administered on the skin, and by 90% when administered in the drinking water. These results indicate an essential role for the products of ODC in TPA promotion of carcinogenesis. (22 Refs)

75. A NEW TUMOR-PROMOTING AGENT, DIHYDROTELEOCIDIN B, MARKEDLY ENHANCES CHEMICALLY INDUCED MALIGNANT CELL TRANSFORMATION.

Hirakawa T, Kakunaga T, Fujiki H, Sugimura T
Lab. Molecular Carcinogenesis, NCI, Bethesda, MD, 20205
Science; 216(4545):527-529 1982

Teleocidin, which was isolated from mycelia of Streptomyces, is a potent tumor promoter in mouse skin. The catalytically hydrogenated compound dihydroteleocidin B markedly enhanced cell transformation induced by 3-methylcholanthrene or ultraviolet radiation. Dihydroteleocidin B was at least 100x more effective in enhancing transformation than 12-O-tetradecanoyl phorbol-13-acetate, the strongest promoter known until now, whereas both promoters showed equal capacities to induce early membrane effects and DNA synthesis. (Author abstract) (24 Refs)

76. GANGLIOSIDE CHANGES INDUCED BY TUMOR PROMOTERS IN PROMOTABLE JB6 MOUSE EPIDERMAL CELLS: ANTAGONISM BY AN ANTIPROMOTER.

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JNCI; 68(3):469-473 1982

Since cell surface gangliosides have been implicated in the regulation of cell growth and differentiation and since tumor-promoting phorbol esters produce a number of membrane changes, the possibility was investigated that ganglioside changes may play a role in promotion of transformation in JB6 mouse epidermal cells. The studies showed that tumor-promoting but not non-promoting phorbol esters produced decreased precursor incorporation into trisialoganglioside GT and increased incorporation into disialoganglioside GD1b and an unknown ganglioside, Gx. These ganglioside responses to promoters were antagonized by the antipromoter retinoic acid, thus suggesting that alterations in ganglioside levels may be involved in promotion of transformation. (Author abstract) (34 Refs)

77. INHIBITION OF RESPIRATION BY A PHORBOL ESTER TUMOR PROMOTER IN MURINE CULTURED CELLS.

Backer JM, Boersig MR, Weinstein IB

Div. Environmental Sciences and Cancer Center/Inst. Cancer Res., Columbia Univ., New York, NY, 10032

Biochem Biophys Res Commun; 105(3):855-860 1982

The effect upon mitochondrial function of in vitro exposure of intact murine embryo fibroblasts (C3H 10T1/2) to 12-0-tetradecanoyl phorbol-13-acetate (TPA) was investigated. Nontoxic doses of TPA induced a rapid decrease in mitochondrial respiration in both normal and carcinogen-transformed fibroblasts. Alterations in mitochondrial function may play an important role in both the initiation and tumor promotion phases of chemical carcinogenesis. (18 Refs)

78. IN VITRO CELLULAR EFFECTS OF HEMATOPORPHYRIN DERIVATIVE.

Berns MW, Dahlman A, Johnson FM, Burns R, Sperling D, Guiltinan M, Siemens A, Walter R, Wright W, Hammer-Wilson M, Wile A

Dept. Developmental and Cell Biology and Surgery, Univ. California, Irvine, CA, 92717

Cancer Res; 42(6):2325-2329 1982

Several in vitro cell systems were exposed to hematoporphyrin derivative (HPD): established lines of rat kangaroo epithelial kidney; normal mouse embryonic fibroblasts; and differentiated neonatal rat myocardial cells. The uptake of HPD (25 to 100 ug/ml) by individual cells occurred rapidly over a 2-hr period and leveled off by 24 hr. HPD was excreted from cells by 48 hr after exposure. However, a low level of HPD (above background) was maintained in cells for up to 4 days following cessation of exposure. Intracellular binding of HPD was to mitochondria as demonstrated by fluorescence microscopy. HPD was also shown to have a growth-inhibiting effect on rat kangaroo cells without added light. The growth effects on mouse cells were less marked. (Author abstract) (18 Refs)

79. SOME PROPERTIES OF THE POLYAMINE DEPRIVATION-INDUCIBLE UPTAKE SYSTEM FOR METHYLGLYOXAL BIS(GUANYLHYDRAZONE) IN TUMOR CELLS.

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Acta Chem Scand [B]; 35(10):731-736 1981

Depletion of the intracellular levels of putrescine and spermidine by the ornithine decarboxylase inhibitor, DL-alpha-difluoromethylornithine (DFMO), resulted in an increased uptake of various polyamines and the spermidine analog methylglyoxal bis(guanylhydrazone) (MGBG) by Ehrlich ascites tumor cells. Examination of the kinetics of uptake of MGBG in DFMO-treated cells showed that polyamine deprivation did not influence the affinity of the transport system, since the Km values were about the same for treated and untreated cells. However, the max velocity for uptake was 4x higher for the polyamine-depleted cells. For both treated and untreated cells, the uptake of MGBG was temperature dependent, with similar values of activation energy of uptake. Ouabain, an inhibitor of Na,K-ATPase, decreased the uptake of DFMO in treated and untreated cells with little effect on cellular ATP, suggesting that the uptake is partially Na linked. While iodoacetate greatly decreased the ATP content of treated and untreated cells, it decreased the MGBG uptake much less than did ouabain, indicating that energy production is not an absolute requirement for drug uptake. In the DFMO-treated cells, 3-4x more MGBG was accumulated in the nuclear, mitochondrial, and cytosol fractions, while the level in the microsomal fraction was 7x higher than in untreated cells. These results may explain the potentiation of the antiproliferative action of MGBG by DFMO. (21 Refs)

80. B16 MELANOMA CELL VARIANTS: IRREVERSIBLE INHIBITION OF GROWTH AND INDUCTION OF MORPHOLOGIC DIFFERENTIATION BY ANTHRACYCLINE ANTIBIOTICS.

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JNCI; 68(4):629-638 1982

The interaction of doxorubicin and N-trifluoroacetyladriamycin-14-valerate (AD32) with B16 melanoma cell variants that exhibit distinct metastatic properties was explored. The addition of the two drugs to cell monolayers at noncytolytic concentrations for 16 hr resulted in irreversible inhibition of proliferation of tumor cells and loss of their tumorigenicity after injection into inbred C57BL/6 mice. Cessation of melanoma cell proliferation was accompanied by cellular and nuclear hypertrophy and the development of axon-like processes. As assessed by drug-specific cytofluorescence, after 16-hr exposure of the cells to doxorubicin and AD32, the drugs were localized in the nuclei. Incubation of drug-treated cell monolayers for 6 additional days in drug-free culture medium led to the complete disappearance of nuclear stain. The highly metastatic B16-F10-B2 cell line was the most sensitive to the drugs' effects, whereas the other two malignant melanoma cell lines, although differing in their metastatic capabilities, exhibited similar sensitivities to the drugs' effects. Doxorubicin was notably more potent than AD32 in its growth inhibitory effect on the three tested melanoma cell variants. The possible mutagenic effect of anthracycline drugs on tumor cells was discussed. (Author abstract) (42 Refs)

81. EFFECT OF CHLORAMBUCIL AND INDOMETHACIN ON GROWTH AND PROSTAGLANDIN LEVELS IN SENSITIVE AND RESISTANT WALKER CARCINOMA.

Tisdale MJ, Gibbs J, Dobrzanski RJ
Dept. Pharmacy, Univ. Aston Birmingham, Gosta Green, Birmingham B4 7ET, England
Chem Biol Interact; 39(2):221-230 1982

An analysis of the effect of combinations of chlorambucil and indomethacin or chlorambucil and prostaglandin E2 (PGE2) on the growth of alkylating agent sensitive and resistant Walker carcinoma in vitro has been made by the isobologram approach. Indomethacin alone acts as a growth inhibitor of the Walker carcinoma. High concentrations of indomethacin (5 ug/ml) act to inhibit the growth of the resistant line sub-additively with chlorambucil, whereas low concentrations act additively. For the sensitive line indomethacin acts either additively or supra-additively with chlorambucil at all concentrations employed. Both indomethacin and low concentrations of chlorambucil alone inhibit PGE2 secretion into the culture medium of both cell lines and an enhanced inhibition is seen with the combination. PGE2 itself acts as a growth inhibitor of both cell lines, although it causes greater growth inhibition of chlorambucil resistant Walker carcinoma (LD50 1.8 ug/ml) than of the sensitive line. This correlates with a greater PGE2 secretion capacity by the resistant cell line (40 pg PGE2/ml medium/10(5) cells for the resistant tumor and 17 pg PGE2/ml medium/10(5) cells for the sensitive tumor). Combinations of PGE2 with chlorambucil inhibit growth either additively or sub-additively. It seems unlikely that inhibition of PGE2 secretion is responsible for the interactive effects of chlorambucil and indomethacin, since growth inhibition produced by the combination is not reversed by PGE2 at any of the concentrations employed. Possible mechanisms of the interactive effects are discussed. (Author abstract) (18 Refs)

82. EFFECTS OF ALKYLATING AGENTS ON THE DNA REPLICATION OF CULTURED YOSHIDA SARCOMA CELLS.

Van Der Steen J, Van Wichen BH, Benckhuysen C
Div. Experimental Chemotherapy and Radiotherapy, Antoni Van Leeuwenhoek-Huis, The Netherlands Cancer Inst., Amsterdam, The Netherlands
Chem Biol Interact; 39(2):191-204 1982

Logarithmically growing Yoshida sarcoma cells were treated for 1 hr with low (2 decades cell kill) or high (more than 6 decades cell kill) doses of alkylating agent. Pulse and chase labeled DNA from treated cells were studied by alkaline sucrose gradient centrifugation. Nitrogen mustard (HN-2), 4-hydroperoxycyclophosphamide (CY-OOH), melphalan (L-PAM) and chlorambucil (CA) had no effect on the elongation rate of newly replicated DNA, both at low and high doses, although per cell the rate of DNA synthesis declined as inferred from the rates of [3H]thymidine incorporation compared to the increase in numbers of S phase cells in the treated populations. It is concluded that these drugs act specifically on the initiation step of the DNA replication, leaving chain elongation undisturbed. At low doses the chemically related sulfur mustard (SM) had also no effect on the maturation of new DNA but at high doses a decreased elongation rate was observed. A transient inhibition of chain growth was observed following treatment with a low dose of 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU). In contrast, the intercalating agent adriamycin showed a severe but delayed effect resulting in an almost complete block of the maturation. (Author abstract) (24 Refs)

83. BIOCHEMICAL AND MORPHOLOGICAL STUDY OF ADRIAMYCIN-INDUCED CHANGES IN MURINE NEUROBLASTOMA CELLS.

Schengrund CL, Sheffler BA
Dept. Biological Chemistry, Milton S. Hershey Medical Center, 500 University Drive, Hershey, PA, 17033
Oncology; 39(3):185-190 1982

Cholinergic murine neuroblastoma cells, maintained in vitro, were exposed to a low concentration (0.4 ug/ml) of adriamycin. Morphologically the treated cells appeared to differentiate. The cell bodies increased in size from an average fixed cell body diameter of 7-13 to 25-40 um, the cells developed long processes, became argyrophilic and the percentage of cells undergoing mitosis decreased relative to controls. Acetylcholine esterase activity increased in the drug-treated cells suggesting induction of differentiation. However, choline acetyltransferase activity and ganglioside composition remained unchanged. In addition, inoculation of mice with 2 x 10(5) viable drug-treated or control cells resulted in all of the mice developing neuroblastoma. No differences were observed in either the rate of tumor development or survival times. These results suggest that neuroblastoma cells may survive adriamycin treatment by becoming 'differentiated', ceasing cell division until conditions favor their undergoing another cell cycle. (Author abstract) (28 Refs)

84. ANTITUMOR EFFECT OF DICYCLOHEXYLAMMONIUM SULFATE, A POTENT INHIBITOR OF SPERMIDINE SYNTHASE AGAINST P388 LEUKEMIA.

Ito H, Hibasami H, Shimura K, Nagai J, Hidaka H
Dept. Pharmacology, Mie Univ. Sch. Medicine, Tsu, Mie 514, Japan
Cancer Lett; 15(3):229-235 1982

The effect of dicyclohexylammonium sulfate (DCHA) on P388 mouse leukemia was investigated; correlation between the antitumor effect and the spermidine concentration in ascites tumor cells and spleen is discussed. Female mice of BDF1 hybrid strain were used. DCHA administered daily for 6 days starting 24 hr after tumor inoculation prolonged the survival time of mice bearing the tumor. The percentage of mean survival days of treated relative to control mice were 110.3, 133.3, 187.2, 138.5, and 118.0 at DCHA dosages of 10, 25, 50, 75, and 100 mg/kg, respectively. A dose of 50 mg/kg/day was the most effective. DCHA was non-toxic at the dose of 100 mg/kg administered daily for 6 days. The treatment of non-leukemic mice with DCHA, 50 mg/kg ip daily for 6 days, had no significant effect on av spleen wt, compared with 0.15 M NaCl treatment. Spleen wt increased by approx 30% at 7 days after the tumor inoculation. DCHA treatment did not prevent the tumor-induced increase in splenic wt. A dose of 50 mg/kg DCHA ip administered daily for 6 days caused a diminution of the increase in concentration of spermidine, with little effect on the spermidine concentration in the ascites tumor cells and spleens of mice bearing the tumor. The depletion of spermidine in the ascites tumor cells might be a cause of the suppression of tumor growth. (26 Refs)

OTHER RELATED STUDIES

85. CELLULAR HETEROCHRONY AND NEOPLASIA.

Pearson RD
Science and Medicine Library, Univ. Toronto, 7 King's College Circle, Toronto, Ontario M5S 1A5, Canada
Med Hypotheses; 8(3):231-235 1982

The concept that 'timing changes' in gene regulation are responsible for malignant transformation and subsequent tumorigenesis is presented. Cells of regenerating rat liver 'retrodifferentiate' (RDF), taking on 'juvenile' or embryonic patterns of antigenic and genetic behavior. RDF is followed by a process of redifferentiation to normal tissue type. Primary malignant cells have often been compared to the invading cells of the trophoblast. Trophoblastic tissue and malignant cells have a number of similarities including their ability to move (metastasize), destroy their host's cells in advance of the invading cells, create a life support system by means of angiogenesis, and subvert their host's immune surveillance and monocyte-macrophage systems. Cellular heterochrony of RDF could explain these similarities. Attempts were made to relate human breast cancer and urogenital cancer to thyroid gland malfunction by showing a higher incidence of cancer in hypothyroidism and a lower incidence in euthyroid and hyperthyroid states. The hormones which render amphibians both neotenic and metamorphic are also possibly responsible for triggering timing changes in the tissues and cells of other metazoans. The observations that the thyroid hormones thyroxine (T4) and triiodothyronine (T3) enhance rat liver regeneration provide indirect evidence to support this hypothesis. T4 or T3 may act as an anticarcinogen. The use of a polar solvent can induce maturation changes in rhabdomyosarcoma transplantable tumor cells from mice and human colon cancer cells causing the cells to lose a marked degree of malignancy. The cancer cells appear to have metamorphosed and thus relinquished their juvenile or heterochronic behavior. (22 Refs)

86. SIGNIFICANT PROGRESS IN DERMATOLOGIC RESEARCH SINCE 1977.

Lapiere CM
Dept. Dermatology, Univ. Liege, B-4020 Liege, Belgium
J Am Acad Dermatol; 6(2):200-208 1982

Recent dermatologic research (1977-1981) of significance for cancer research includes: the identification of the Langerhans cells and partial elucidation of their role in cell-mediated immunity; the typing of mononuclear cells; the identification of T-lymphocyte growth factors; the association of defined subtypes of human papovavirus with neoplastic transformation; continuing research on melanogenesis and its control by polypeptides; the functions of specific proteins of the keratinocytes; the roles of membrane receptors, growth factors, and intracellular enzymes in the control of epidermal cell proliferation and differentiation; and phototherapy/photochemotherapy of cancer. (129 Refs)

87. CHANGES OF ORNITHINE DECARBOXYLASE ACTIVITY AND POLYAMINE CONTENT UPON DIFFERENTIATION OF MOUSE NB-15 NEUROBLASTOMA CELLS.

Chen KY, Presepe V, Parken N, Liu AY
Dept. Chemistry, Rutgers-The State Univ. New Jersey, New Brunswick, NJ, 08903
J Cell Physiol; 110(3):285-290 1982

Specific changes of basal level of ornithine decarboxylase (ODC) activity and spermidine content during the differentiation of murine NB-15 neuroblastoma cells and the temporal relationship of these changes and the expression of differentiated phenotypes of neuroblastoma cells are reported. ODC activity of nondifferentiating (NB) neuroblastoma cells and differentiating (ND) cells was studied over a 5 day period. NB cells showed an early increase in ODC activity that was temporarily associated with the resumption of logarithmic growth after plating. Upon entering the stationary phase of cell growth, ODC activity in NB cells decreased precipitously, reaching a minimum activity of 0.2 units/mg protein at the end of the 5 day culture period. In ND cells, the early increase in ODC activity was attenuated in both magnitude and duration. The increase in ODC activity of ND cells was transient. While putrescine levels were similar in NB and ND cells. Significant differences were observed in levels of cellular spermidine. Spermidine content of NB cells followed a steady increase from the basal level of 1.5 nanoMols (nM)/mg protein to a maximal value of 5.05 nM/mg protein. This elevated level of spermidine in NB cells was sustained for the entire 5 day period. The significance of cAMP-induced changes in ODC activity and polyamine contents in cell differentiation are discussed. (15 Refs)

88. ORNITHINE DECARBOXYLASE ACTIVITY IN CHEMICALLY INDUCED MOUSE SKIN PAPILLOMAS.

Astrup EG, Boutwell RK
Inst. Pathology, Univ. Oslo, Rikshospitalet, Oslo 1, Norway
Carcinogenesis; 3(3):303-308 1982

The activity levels of L-ornithine carboxy-lyase (ODC) (EC 4.1.1.17) and S-adenosyl-L-methionine carboxy-lyase (SAM-D) (EC 4.1.1.50) were determined in individual papillomas induced in mouse skin by a two-stage technique, and in normal mouse epidermis. Cycloheximide treatment abolished both enzyme activities. In normal epidermis the ODC activity was barely detectable, whereas the tumors exhibited high levels of ODC. Levels of SAM-D activity above those of normal epidermis were detected in some papillomas, but in contrast to ODC the SAM-D activity levels were not consistently increased in skin tumors. By pooling a great number of papillomas, the variations in ODC and SAM-D activities between different papillomas could be minimized so that reliable measurements of the biological half-lives of ODC and SAM-D in the tumors were obtained using cycloheximide treatment. The half-life of SAM-D in squamous papillomas was 45 min, almost identical to the 41 min half-life of the 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced level of this enzyme in normal mouse epidermis. In contrast, the ODC activity of the mouse skin papillomas declined at a rate similar to that in TPA-treated epidermis for only the first 15-20 min after cycloheximide injection. Thereafter, at time points when protein synthesis was approx 90% inhibited, the ODC activity reverted to high levels. These results show that the high level of ODC activity in squamous papillomas is stabilized. This observation is compatible with the hypothesis that the control mechanism of the ODC activity level in these tumors is severely deranged. This change in polyamine turnover pattern may be related to altered differentiation of the epidermal cells, which constitute the main bulk of cells in these tumors. (Author abstract) (40 Refs)

89. SERUM-FREE MEDIUM FOR THE IN VITRO GROWTH OF NORMAL AND MALIGNANT URINARY BLADDER EPITHELIAL CELLS.

Messing EM, Fahey JL, DeKernion JB, Bhuta SM, Bubbers JE (c/o Bubbers) Dept. Surgery, Univ. California Los Angeles Sch. Medicine, Los Angeles, CA, 90024
Cancer Res; 42(6):2392-2397 1982

A serum-free medium, DH-S1, is described which is valuable for the establishment of primary cultures of normal and malignant transitional epithelium (transitional cell carcinoma of the bladder). Growth of epithelial cells in DH-S1 is facilitated but that of fibroblasts is suppressed. Another established human transitional cell carcinoma cell line, 647V, has grown continually in DH-S1 for over 36 passages. Morphological and antigenic studies comparing 647V cells growing in serum-containing and serum-free medium reveal differences which are pronounced at low cell density but which almost disappear at higher densities. A new human transitional cell carcinoma cell line, LA-B1, is described whose initial growth was supported by this serum-free medium. (Author abstract) (22 Refs)

90. DEGREES OF MALIGNANCY IN HUMAN PRIMARY CENTRAL NERVOUS SYSTEM TUMORS: ORNITHINE DECARBOXYLASE LEVELS AS BETTER INDICATORS THAN ADENOSYLMETHIONINE DECARBOXYLASE LEVELS.

Scalabrino G, Modena D, Ferioli ME, Puerari M, Luccarelli G Inst. General Pathology, Univ. Milan, Via Mangiagalli 31, 20133 Milan, Italy
JNCI; 68(5):751-754 1982

The levels of activity of ornithine decarboxylase (ODC) and adenosylmethionine decarboxylase (AMD) were measured in various types of primary human tumors of the central nervous system (CNS) and whenever possible were related to the malignancy of the tumor graded according to histopathologic criteria. In astrocytomas ODC levels increased linearly and progressively from infratentorial pilocytic astrocytoma (Grade 1) t glioblastoma multiforme (Grade IV) and correlated well with the degree of histologic malignancy of the tumor. AMD activity levels, however, correlated with tumor malignancy only up to Grade III astrocytoma. Medulloblastomas exhibited an unusual dichotomy with regard to the levels of polyamine biosynthetic decarboxylases (PBD): Medulloblastomas had the highest ODC activities of all the CNS tumors tested but had low AMD activities. In tumors of neuroepithelial tissue ODC level increases and, when present, AMD level increases were not due to proliferation of new blood vessels, because CNS hemangioblastomas had very low levels of both PBD activities. No significant differences in either of the PBD levels were observed among the several variants of meningiomas tested, the meningotheliomatous, the transitional, and the fibrous meningiomas. However, atypical forms of meningioma, ie those with mitotic figures, whatever the histologic variants, had higher levels of ODC, but not of AMD, than the typical forms, ie, those without mitotic figures. (Author abstract) (28 Refs)

91. CELL GROWTH AND TRNALYS4 SYNTHESIS IN MOUSE 3T3 CELLS.

Ortwerth BJ, Wolters J, Nahlik J, Conlon-Hollingshead C Dept. Ophthalmology, Univ. Missouri, Columbia, MO, 65201
Exp Cell Res; 138(2):241-250 1982

The RPC-5 chromatographic profiles of lys-tRNA were analyzed during the growth of 3T3 cells in culture. An inverse relationship was seen between tRNAlys2 and tRNAlys4 which was markedly influenced by medium changes. This interchange of tRNAlys2 and tRNAlys4 could be controlled by altering the levels of serum in the medium, or more precisely by altering the serum to cell ratio. A different change in lys-tRNA distribution was seen when the cells reached confluency. The amounts of tRNAlys2, tRNAlys3 and tRNAlys4 all decreased with a corresponding increase in either tRNAlys5 or tRNAlys6. An identical change in lys-tRNA could be produced by shifting sparse cells into a medium containing 10% calf plasma instead of 10% serum. Both tRNAlys profiles and cell growth were returned to normal when the cells were returned to medium with 10% fetal calf serum (FCS) or 10% calf plasma and fibroblast growth factor (FGF). A third alteration in tRNAlys profiles was seen by the addition of cAMP to the cultures. A decrease in tRNAlys5 and a corresponding increase in tRNAlys6 was seen upon the addition of 10(-3) M db-cAMP and was accentuated by the simultaneous addition of 10(-3) M methyl isobutylxanthine. These data are consistent with an ordered sequence of tRNAlys modification involving tRNAlys2, tRNAlys3, tRNAlys4, tRNAlys5B and tRNAlys6. Several of the factors which control proliferation appear to control the activity of different tRNA-modifying enzymes in this tRNAlys pathway thereby controlling the levels of tRNAlys4, a tRNA previously shown to correlate directly with the proliferative rate of cells. (Author abstract) (23 Refs)

92. MACROPHAGE ACTIVATION FOR TUMOR CYTOTOXICITY: REACTIVITY OF PERITONEAL AND BONE MARROW MACROPHAGES.

Ruco LP, Meltzer MS, Leonard EJ, Tomisawa S, Gallily R, De Chatelet LR, Patriarca P, Clark RA, Rossi F
Second Inst. Pathological Anatomy, Univ. Rome, Viale Regina Elena 324, 00161 Rome, Italy
Adv Exp Med Biol; 141:85-97 1982

Various studies concerning tumor cytotoxicity induced by murine (CIMLAM) lymphokine-activated macrophages are reported. The following aspects of CIMLAM are included course of lymphokine activation (LA), effect of time in culture prior to LA, lymphokine responsiveness of resident and inflammatory macrophages, and effect of x-irradiation. Peritoneal macrophage function was also studied during acute inflammation. Reactive cells differentiated to a non-reactive stage within 24 hr and either in vivo BCG activated or in vitro lymphokine activated macrophages maintain their status for a few hr. The short-lived nature of both macrophage tumoricidal capacity and the capacity of precursor cells to be activated by lymphokines may function as negative feedback mechanisms in immune reactions. The close association found between the frequency of macrophages with intracytoplasmic peroxidase-positive granules and lymphokine reactivity of the cell population may indicate that lysosomal peroxidase may be involved in lymphokine activation for tumor cytotoxicity. (11 Refs)

93. PRINCIPLES OF CANCER BIOLOGY: CELL BIOLOGY AND BIOCHEMISTRY OF CANCER.

Pardee AB
Div. Cell Growth and Regulation, Signey Farber Cancer Inst., Harvard Medical Sch., Boston, MA
Cancer - Principles and Practice of Oncology. DeVita VT, Hellman S, and Rosenberg SA, ed. Philadelphia, Lippincott, 1926 pp., 1982.

No differences between malignant and nonmalignant cells have yet been discovered which can serve as a basis for a universally accepted therapy. Recent research in cell biology, tissue culture, biochemistry, and cell genetics may help to provide answers for the somewhat more specific questions. Ques-

tions such as: growth factors required by normal and neoplastic cells, the biochemical mechanisms which determine cell number increases or non-growth, the numbers and chromosomal locations of 'cancer' genes, the cause(s) and significance of the rapid appearance of chromosomal changes in cancer cells, the basis of anaplastic morphologic and biochemical differentiation in cancer cells, the roles of surface, membrane, and cytoplasmic structure changes in neoplasia, and the role of error-prone DNA repair in tumorigenesis and drug resistance, can now be asked. Current cancer therapy is an attempt at selective cell killing, based upon the greater growth fraction of cancer cell populations. It is possible that in the early stages of tumor progression, the difference between tumor-forming and non-tumor-forming cell populations may be small and quantitative. Further research is needed to define these differences and exploit them for treatment and prevention. (153 Refs)

94. THE PATHOGENESIS OF PLASMA CELL TUMORS.

Hollander VP, Platica M

Res. Inst., Hospital for Joint Diseases, New York, NY, 10035

The Handbook of Cancer Immunology Volume 6: Lymphoid Cell Subpopulations-Structure, Function, and Interactions. Waters H, ed. New York, Garland STPM Press, 314 pp., 1981.

The possibility is examined that common factors are at play in the mineral-oil induced plasma cell tumors (PCT) in BALB/c mice because so many endocrine factors appear to modulate tumorigenesis. The working hypothesis involves bacterial lipopolysaccharide in a transformation step, pristane-induced peritoneal factors in the successful adaptation of nascent tumor cells to the host, and finally, an immunosuppression step which permits rapid growth. Hormones are involved in the earlier steps of plasmacytoma development. (145 Refs)

95. ANTAGONISM OF CONCANAVALIN A CAPPING IN PHORBOL ESTER-ACTIVATED LYMPHOCYTES BY CALMODULIN INHIBITORS AND CERTAIN AMINO ACID ESTERS.

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(c/o Mueller) McArdle Lab. Cancer Res., Univ. Wisconsin, Madison, WI, 53706

Cancer Res; 42(6):2115-2120 1982

The compound 12-O-tetradecanoylphorbol-13-acetate (TPA), an effective tumor promoter in mouse skin and comitogen in bovine lymphocytes, rapidly stimulates concanavalin A-mediated cap formation in the latter cells. The ability of different phorbol derivatives to facilitate the capping reaction correlates well with their potencies as lymphocyte comitogens and as tumor-promoting agents. This effect of TPA on capping in bovine lymphocytes, which is apparent within min, is neither mimicked nor altered by dibutyryl cyclic adenosine 3':5'-monophosphate or cyclic guanosine 3':5'-monophosphate. Cytochalasin D, a microfilament-disrupting agent, inhibits cap formation, thereby suggesting the participation of microfilaments in the response. benzoyl tyrosine ethyl ester selectively inhibits the TPA-stimulated cap formation, whereas benzoyl tyrosinamide is inactive. A comparison of related amino acid derivatives reveals that their activities are dependent on the nature of both the amino acid side chain and the carboxyl end blocking group. Trifluoperazine and N-(6-aminohexyl)-5-chloronaphthalenesulfonamide, known inhibitors of the calmodulin-dependent processes, also selectively block the TPA-stimulated cap formation, whereas trifluoperazine sulfoxide, a less effective calmodulin antagonist, is relatively inactive. These data suggest that the stimulation of capping by TPA involves the activation of a calmodulin-dependent process which may also be regulated by the function of an esterase. (Author abstract) (32 Refs)

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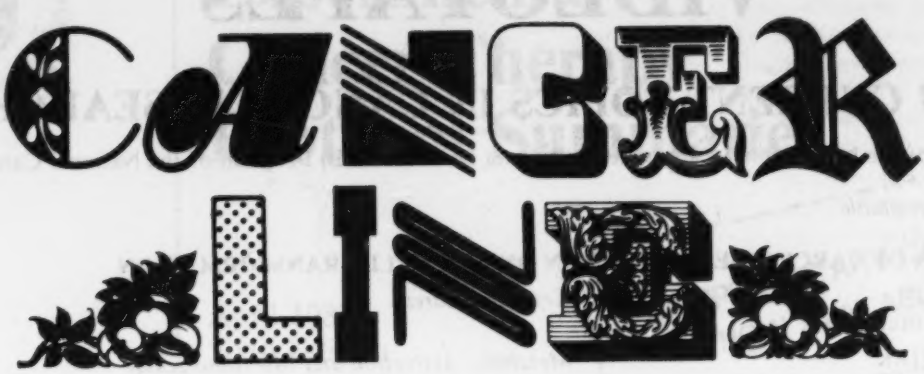

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Growth Regulation, Differentiation

SERIES CB16 No. 82/10 OCTOBER, 1982

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CELL AND SERUM DERIVED GROWTH EFFECTORS

1. EFFECT OF MONOCLONAL ANTI-TYROSINE HYDROXYLASE ANTIBODIES ON MELANOMA CELL DIFFERENTIATION (MEETING ABSTRACT)

Hakim AA, Joseph CE
University of Illinois at the Medical Center, Chicago, IL
Hybridoma; 1(2):195 1982

Tyrosine hydroxylase (TH) is a key enzyme in melanogenesis. The present studies examined the biological effects of Hy' on melanotic melanoma cell lines. Three melanoma cell lines were examined for responsiveness to estrogen, binding of estradiol, TH-activities and melanin biosynthesis. Aliquots of each were cultured in parallel in (A) DMEM (Dulbecco's minimum essential medium), and (B) DMEM supplemented with the monoclonal anti-tyrosine hydroylase Hy-7'. Both cultures were incubated in the same incubator with 5% CO2 humidified sterile air, fed at the same time and with the same volume of their nutrients, and they were split at the same time and at the same proportion. Both cell cultures maintained approximately the same doubling time. The (A)-cultures remained melanotic and

HE 20.3173/2:

responsive to estrogen. Their cytosols maintained estradiol-binding affinity. The (B) cultures became amelanotic, non-responsive to estrogens and their cytosols did not bind estradiol, and had statistically non-significant tyrosine hydroxylase activity. Scatchard analysis of estradiol binding of cytosols from cultures of mammary epithelial (ME), mammary carcinoma (mCa) and malignant melanotic melanoma (MMM) cells showed that Hy-7' inhibited estrogen binding to cytosols from (MMM) cells. Hy-7' had no effect on estrogen binding to cytosols of either EM or mCa cells. Hy-7' antibodies inhibited melanin synthesis in MMM cells. It had no effect on melanin synthesis in normal pigmented epithelial cells. (1 Ref)

2. GROWTH FACTORS (GF) AND TRANSITIONAL CELL CARCINOMA (TCC): THEIR INTERACTION EVALUATED WITH THE AID OF A SERUM-FREE DEFINED MEDIUM (MEETING ABSTRACT)

Messing EM, Fahey JL, DeKernion JB, Bubbers JE
No affiliation given
77th Annual Meeting of the American Urological Association, Inc., May 16-20, 1982, Kansas City, Missouri. American Urological Association 254 pp., 1982.

For our investigations of the production of new growth factors by TCC, 647V was chosen as the most appropriate subject not only because of its ability to grow in DH-S1, but also because its growth in both serum-containing and serum-free media has demonstrated a rigid dependency upon cell density. Concentrated (Amicon Filter, 500MW cut-off) conditioned serum-free media, obtained from 647V cultures in log phase growth in DH-S1, was then added to fresh DH-S1 and was found to stimulate growth of 647V cells in a direct, dose-related fashion. When this activity was compared with that of the ubiquitous epidermal growth factor (EGF), itself a naturally occurring GF which is excreted in large quantities in a biologically active form in human urine, and which has demonstrated co-carcinogenic properties, EGF failed to stimulate the growth of 647V cells when added to growth media (DH-S1) in several concentrations. Subsequent experiments which detected and measured EGF receptors on 647V and another human TCC cell line, showed that the inability of EGF to stimulate growth of these cells was not because EGF was not bound by them. Tumors characteristically are less responsive than non-neoplastic tissue to normal growth regulation. The persistance of GF production by a cell which is capable of responding to that factor by dividing may result in continuous endogenous stimulation and eventually to unrestricted growth. (no Refs)

3. OAT-CELL CARCINOMA WITH FIBROSIS. EVIDENCE FOR RELEASE OF A GROWTH SUBSTANCE.

Newcom SR, O'Rourke L
Section Medical Oncology, Oregon Health Sciences Univ., 3181 S. W. Sam Jackson Park Road, Portland, OR, 97201
Cancer; 49(11):2358-2364 1982

Recent work demonstrates that transformed cells can produce mitogenic agents capable of stimulating fibroblast proliferation. Also, work with normal cells and tissue has recently demonstrated that, among other cells, macrophages and platelets contain and elaborate potent mitogens capable of stimulating fibroblast proliferation. An unusual oat-cell carcinoma associated with large amounts of fibrous tissue was studied. Short-term cell culture of the pleural fluid was utilized to measure the production of a growth substance. Macrophages were depleted by surface adherence. A potent conditioned medium was obtained, which induced proliferation of BALB/c 3T3 cells and human diploid fibroblasts. This conditioned medium, renewed weekly, was at least as potent as Fibroblast Growth Factor 100 ng/ml every other day. It is concluded that in this human malignancy fibroblast proliferation surrounding the malignant cell is secondary to production of mitogens by the malignant cell. (Author abstract) (23 Refs)

4. CELL DIVISION AND CHANGES OF THE DURATION OF MITOSIS IN MICE EHRLICH ASCITES CARCINOMA AFTER SINGLE ADMINISTRATION OF CHALONE-CONTAINING EXTRACT FROM THIS TUMOR.

Romanov IuA, Semenova MV
Dept. Biology, N. I. Pirogov Second Medical Inst., Moscow, USSR
Biull Eksp Biol Med; 93(3):81-83 1982

The effect of chalones on kinetics of cell division was studied in random-bred albino mice bearing Ehrlich ascites carcinoma (EAC). The mice with 5-day EAC were injected with chalone-containing extract from the 13-day EAC. Analysis of the mitotic activity (MA) showed that chalone induced phase changes in cell division. The inhibition of MA was associated with the arrest of cells in phase G2. Stimulation phase was associated with cell synchronization, and the normalization phase was associated with physiological regulation of cell division. (11 Refs) (In RUS)

5. DETERMINATION OF EPIDERMAL G2-CHALONE IN EPIDERMOID TISSUES.

Simbirtsev AS
Lab. Experimental Histology, Res. Inst. Experimental Medicine, Leningrad, USSR
Arkh Anat Gistol Embriol; 82(2):100-106 1982

The presence of epidermal G2-chalone was studied in normal epidermoid tissues. The tissue specimens were treated by monospecific rabbit immune serum against rat G2-chalone and assayed by the indirect immunomorphological Coon's technique. G2-chalone was detected in the epithelium of the cornea, bladder, kidney pelvis, and thymus. G2-chalone was absent in the stroma of the lymph nodes and spleen. G2-chalone could not be detected in the tracheal epithelium. (16 Refs) (In RUS)

6. AN INHIBITOR OF DNA-SYNTHESIS, ISOLATED FROM RAT TUMOR-INDUCED ASCITES.

Wallevik K, Jensenius JC, Larsen JK, Kroll J, Lassota H
Finsen Lab., Finsen Inst., 49, Strand Blvd. 24/2, DK-2100 Copenhagen, Denmark
Virchows Arch [Cell Pathol]; 39(2):145-158 1982

An inhibitor of DNA-synthesis in rat Yoshida hepatoma cells in vitro was isolated from the corresponding ascites tumor by phosphocellulose chromatography. The inhibition is non-specific as the isolated fraction also inhibited normal rat thymus and spleen cells as well as other hepatomas and rat Walker carcinoma. Fractions isolated from Walker ascites and pooled normal rat serum have inhibitory properties similar to the inhibitor fractionated from Yoshida ascites by the same procedures. The inhibitory activity isolated from rat serum is significantly less than the one isolated from the Yoshida ascites. The cell lines examined showed gross variation in sensitivities to the inhibitor. This is, however, a characteristic of the cell rather than the inhibitor. The demonstrated variations in the responses among the cell lines to one inhibitor brings seriously into question the tissue-specificity of many growth inhibitors claimed to possess this property. The present findings emphasize the requirement for tests of tissue specificity of an inhibitor of cell

growth: (1) The growth of at least three cell types from tissues different from the one in question must be shown to be unaffected by the inhibitor, and most important, (2) A fraction isolated from a different tissue by exactly the same procedure must leave the original cells uninhibited whether or not it inhibits the cells of the donor tissue. The inhibitor isolated from rat ascites and serum delays the initiation of the DNA-synthesis in resting cells that are stimulated to regrowth. It has an apparent mol wt of around 100,000 daltons, is relatively resistant to heat (100 C), but is destroyed by subtilisin. (Author abstract) (17 Refs)

7. CALMODULIN AND CA2+ IN NORMAL AND TRANSFORMED CELLS.

Vsigl ML, Sedwick WD, Vanaman TC
Dept. Microbiology and Immunology, Duke Univ. Medical Center, Durham, NC, 27710
Fed Proc; 41(7):2283-2288 1982

Numerous lines of evidence implicate calcium and calmodulin (CaM) as regulators of cell growth and functional differentiation. In light of this evidence, several studies of the possible involvement of the CaM system in cellular transformation by RNA and DNA tumor viruses have been carried out. This paper summarizes the evidence linking calcium and CaM to the regulation of cell growth and critically examines the evidence that increases in CaM levels occur in transformed versus normal cells. A nontraumatic method for synchronizing both normal and transformed chick fibroblasts is presented. This method is utilized in a comparison of CaM levels throughout the cell cycle of Rous sarcoma virus transformed and normal chick embryo fibroblasts. These studies best support the hypothesis that the observed differences in CaM levels between transformed and normal cultures under optimal growth conditions may largely reflect differences in the proportion of cells in a dividing versus a nondividing state. (Author abstract) (41 Refs)

8. PERMISSIVE EFFECT OF HUMAN COLONIC CARCINOMA CELLS (HT-29 AND CACO-2) ON THE DIFFERENTIATION OF INTESTINAL SMOOTH MUSCLE.

Haffen K, Simon-Assmann P, Kedinger M, Grenier JF, Zweibaum A
Physiopathologie digestive, Unite de Recherche 61 de l'I.N.S.E.R.M., 3, avenue de Moliere, 67200 Strasbourg-Hautepierre, France
C R Acad Sci [III] (Paris); 293(15):807-812 1981

The permissive effect of human colonic carcinoma cells (lines HT-29 and Caco-2), associated with 5.5-day-old chick embryo intestinal mesenchyma and grafted into 3-day-old chick embryos for 11 days, was studied. The growth of these grafts was considerable compared with that of pure tumor cell grafts not associated with chick embryo intestinal mesenchyma. The histological examinations performed after grafting of associated carcinoma cells revealed the presence of differentiated muscular layers around the cancer cells. The carcinoma cells were found to provoke the differentiation of the intestinal mesenchyma into muscular layers, even though this mesenchyma is incapable of undergoing autonomous differentiation. The results provide additional evidence that HT-29 and Caco-2 cells exhibit properties common to fetal and normal intestinal cells. (22 Refs) (In FRE)

9. A DIFFERENCE IN THE GROWTH RESPONSE OF CAPILLARY AND AORTIC ENDOTHELIAL CELLS TO TUMOUR ANGIOGENESIS FACTOR (TAF) (MEETING ABSTRACT)

Brierley AL
Christie Hospital, Manchester, England
Br J Cancer; 44(2):310 1981

Capillary-derived and aorta-derived endothelial cells were grown on plastic dishes and native collagen gels. Addition of TAF to these cultures and examination of subsequent cell numbers revealed: (a) Capillary cells growing on native collagen gels were stimulated to proliferate by addition of TAF; (b) No stimulation was observed for either aorta-derived cells growing on plastic or native collagen gels, or for capillary cells on a plastic substrate. This data suggests a functional difference between large and small-vessel endothelial cells, a difference which has wide implications when undertaking studies on diseases involving endothelial-cell disorders. (no Refs)

10. STIMULATION OF GROWTH IN SOFT AGAR OF SW-13 HUMAN CARCINOMA CELLS BY FACTORS RELEASED BY THESE CELLS AND BY EXTRACTS FROM NORMAL AND NEOPLASTIC HUMAN TISSUES (MEETING ABSTRACT)

Halper J, Moses HL
Departments of Cell Biology and Pathology, Mayo Clinic Foundation, Rochester, MN, 55905
Fed Proc; 41(3):854 1982

SW-13 cells, derived from a human adenocarcinoma of the adrenal, form a few small colonies when cultured in soft agar at low cell densities. The number and size of colonies increased dramatically following stimulation with serum-free medium conditioned by SW-13 cells, suggesting the possibility of autostimulation in these malignant cells. Both an acid/ethanol extract and serum-free conditioned medium from A431 cells (human squamous carcinoma cells) caused up to a 30-fold increase in the number of colonies formed by SW-13 cells. Acid/ethanol extracts from a variety of freshly excised human malignant neoplasms were tested for stimulation of SW-13 cell colony growth. Overall, extracts from 21 of 31 carcinomas stimulated colony growth by three to 90-fold. Extracts from seven of the eight adenocarcinomas of the colon caused stimulation. No stimulation was obtained from three sarcomas, two melanomas and a chordoma. Extracts from nonneoplastic human kidney and lung caused stimulation of colony formation up to 20-fold. All of the colony stimulating activity was nondialyzable. Preliminary data suggest the human SW-13 cells respond to factors(s) derived from epithelial neoplasms and tissues, but not from nonepithelial neoplasms. The relationship of these factors(s) to known growth factors including the transforming growth factors remains to be established. (no Refs)

11. ISOLATION OF A FACTOR FROM EHRLICH ASCITES CARCINOMA FLUID WHICH PROMOTES DNA SYNTHESIS IN MOUSE LIVER AND PAROTID SALIVARY GLAND (MEETING ABSTRACT)

Burns ER, Yeh HW, Yeh YC
College of Medicine, University of Arkansas for Medical Sciences, Little Rock, AR, 72205
Fed Proc; 41(3):497 1982

A factor(s) which stimulates in vivo DNA synthesis in both the liver and the parotid salivary gland of the adult mouse has been isolated from the acellular ascitic fluid of the Ehrlich as-

cites carcinoma. This factor(s) has been purified about 5,000 fold and is biologically effective at a protein concentration of 3 ug per 25 gm body weight. The purification procedure included ammonium sulfate fractionation and DEAE (diethylaminoethyl) Sephacel column chromotography. This potent, heat labile mitogen is presumed to be a protein with a molecular weight of between 3,500 and 13,000 daltons. Hypophysectiomzed mice responded to the stimulatory effect of this factor(s), indicating that the pituitary gland is not directly involved in mediating the effects of this mitogen. The biochemical properties of this substance as well as the kinetics of the DNA synthetic response are significantly different from those found in isoproterenol and epidermal growth factor stimulated systems. (no Refs)

12. CHARACTERIZATION OF HYPOTHALAMIC ORIGIN GROWTH FACTOR(S) FOR RAT PITUITARY TUMOR CELLS IN CULTURE (MEETING ABSTRACT)

Sirbasku DA, Moo JB
The University of Texas Medical School, Houston, TX, 77025
Fed Proc; 41(3):489 1982

Several hypothalamic hormones have been characterized which are delivered via the portal circulation to the anterior pituitary regulating pituitary hormone production. One of these hormones, TRH (thyrotropin-releasing hormone), has been implicated in the growth response of normal and neoplastic pituitary cells. To further explore the hypothalamus for additional activities that may participate in the regulation of pituitary growth, we have utilized a subclone (GH3/C14) of the original GH3 cell line as a sensor system for probing hypothalamic extracts. Although these cells cannot be used to study all of the physiological functions of the normal pituitary, they do respond to physiological concentrations of estrogens, glucocorticoids, thyroid hormones and TRH in a qualitatively similar manner to cultures of normal cells. Studies with extracts of various separated areas of the rat brain showed that hypothalamic extracts caused a sustained increase in cell number when added to serum-free medium, whereas cerebellum, cerebrum and brain stem extracts were inactive. The rat hypothalamic activity is stable in 1% acetic acid, and at neutral PH has an apparent molecular weight of 25,000 daltons. Large scale purification appears possible using an acid extract of ovine hypothalamus and modified assay procedure requiring only 10 hours, exposure to growth factor. (no Refs)

13. DETECTION OF RAT PROSTATE STROMAL AND EPITHELIOID CELL GROWTH INHIBITORS IN ACID-ALCOHOL OR CHLOROFORM-EXTRACTED DUNNING ADENOCARCINOMA AND IN A METASTATIC HUMAN COLON CANCER (MEETING ABSTRACT)

Rubenstein M, Bonomi P, Anderson KM
Department of Medicine, Rush Medical College, Chicago, IL
Proc Am Assoc Cancer Res; 23:934 1982

When Sprague-Dawley ventral prostate stromal (band 2) or epithelial (band 4) cell-enriched fractions were cultured in RPMI containing insulin, vitamin A, and 20% fetal calf serum, predominantly stromal cells appeared in fraction 2 and initially epithelioid cells, subsequently accompanied by stromal cells in fraction 4. When the culture medium contained a 10% extract in PBS+0.1% EDTA from a Dunning R-3327 rat prostate adenocarcinoma, subline G (Miami), stromal growth in both fractions was suppressed, while epithelioid clones accompanied by a second population of triangular-shaped cells grew in fraction 4. If the Dunning extract was itself extracted with CHCl3, stromal cells in fractions 2 and 4 were still suppressed, while the growth of epithelioid cells and the second unidentified cell population in 4 was enhanced. Addition of an extract prepared from acid/alcohol-extracted Dunning cancer increased the growth of fibroblasts and presumably other stromal cells in both fractions. When a hepatic metastasis from a human colon cancer served as the source of CHCl(3)-treated cancer extracts, incubation with prostate fraction 4 from a Copenhagen x Fisher F1 rat duplicated these effects on stromal and epithelioid cell growth. (1 Ref)

14. ISOLATION AND CHARACTERIZATION OF A NOVEL GROWTH FACTOR (EMBRYONIN) FROM FETUIN (MEETING ABSTRACT)

Salomon DS
Laboratory of Pathophysiology, National Cancer Institute, National Institutes of Health, Bethesda, MD, 20205
Proc Am Assoc Cancer Res; 23:929 1982

Fetuin is an acidic, alpha-globulin glycoprotein (Mw, 50,000; pI, 4.1) found in fetal calf serum at a concentration of 10-20 mg/ml. Pedersen-prepared fetuin is relatively heterogeneous and has been utilized as a medium supplement for growing a variety of cell types in serum-free, hormone-supplemented media. Homogeneous preparations of fetuin prepared by the methods of Deutsch or Spiro lack this growth-promoting activity. An attempt has been made to isolate and characterize this apparent contaminant from Pedersen fetuin. A mouse embryonal carcinoma (EC) cell line was utilized to test for this activity since these cells exhibit an obligatory requirement for Pedersen fetuin for growth in the absence of serum. Pedersen fetuin was fractionated by carboxymethycellulose (CMC) chromatography. A gradient of ammonium formate, pH 5.2 (0.01-1.OM) was used to elute the growth-promoting activity which was recovered from the column at 0.15M salt. This material was rechromatographed on a CMC column and the 0.15M salt eluate was concentrated, dialyzed and chromatographed on a high-pressure liquid chromatography (HPLC) molecular sieve column in 0.2M ammonium formate (pH 5.2). A peak of growth-stimulating activity was recovered from the high molecular weight region of the HPLC column. Analysis of this material revealed a single protein band of approximately 180,000 daltons. The 180K protein (embryonin) stained with Stains All suggesting that it was a glycoprotein. Preparative isoelectric focusing demonstrated a single peak of growth-promoting activity with a pI of 6.8. 20-50 ug/ml of embryonin was found to be optimal for promoting EC cell growth and could completely replace the requirement for Pedersen fetuin in the absence of serum. (no Refs)

15. THE EFFECT OF LIVER CYTOSOL ON HEPATIC REGENERATION AND TUMOR GROWTH (MEETING ABSTRACT)

Makowka L, Falk RE, Falk JA, Teodorczyk-Injeyan J, Venturi D, Falk W, Langer B, Blendis L, Phillips MJ
Department of Surgery, University of Tororto, Toronto, Ontario, Canada
Joint Meeting of the Society of Surgical Oncology and Society of Head and Neck Surgeons, April 18-23, 1982, Marco Island, Florida. The Society of Surgical Oncology and the Society of Head and Neck Surgeons 265 pp., 1982.

This report describes the effect of liver cytosol (100,000g supernatant), both normal (NLC) (from intact adult rat liver) and regenerating (RLC) (from adult rat liver remnants 24 hrs after 68% hepatectomy) on post hepatectomy liver regeneration in the rat, and on tumor growth (1) methylcholanthrene induced bladder carcinoma (FBCa) in Fischer rats, 2) HTC hepatoma

in Buffalo rats and 3) P815 mastocytoma in DBA/2J mice) to determine tissue specificity. Male Lewis rats underwent 68% hepatectomy (PH) and were randomly divided into 3 groups; I Control (homogenization buffer), II NLC (13 mg/ml total protein), III RLC (15 mg/ml total protein). Treatment (5ml/rat) was administered IP 3 hrs post PH. Hepatic incorporation of 3H-thymidine (3H-TdR) was measured for up to 52 hrs post PH. NLC (II) resulted in approximately 70-80% inhibition of the peak 3H-DNA specific activity seen in controls at 18 and 24 hrs post PH. RLC after PH significantly increased the rate of regeneration when compared to PH alone at 24 hrs. Autoradiographic determination of hepatic nuclear labelling confirmed this. Syngeneic NLC or RLC at 6 and 24 days after subcutaneous tumor inoculation resulted in significant inhibition of tumor growth for both FBCa and HTC when compared to control. The retardation of FBCa growth could be further enhanced by administering cytosol every 3 or 7 days. Syngeneic mouse cytosol (12 mg/ml, 1 ml IP/mouse) also significantly inhibited SC P815 matocytoma growth compared to control. Proliferation of P815 cells in vitro was significantly inhibited in cultures in the presence of mouse or rat cytosol as measured by the incorporation of 3H-TdR and cell count. This effect was both time and dose-dependent with greatest inhibitory activity observed at concentrations ranging from 5-1% and at 24-72 hrs after culture initiation. (no Refs)

16. PURIFICATION AND PARTIAL CHARACTERIZATION OF ANTITUMOR GROWTH FACTOR(S) ISOLATED FROM NORMAL HUMAN CELLS (MEETING ABSTRACT)

Martinez AS

St. Pius X High School, Albuquerque, NM

148th National Meeting of the American Association for the Advancement of Science, January 3-8, 1982, Washington, D.C. American Association for the Advancement of Science 182 pp., 1982.

In a previous study, I reported the presence of a substance in conditioned medium and whole-cell extracts from normal human cells which inhibited the growth of tumor cells. The objective of the present study was to purify, characterize, and attempt to identify the factor(s) by affinity chromatography and polyacrylamide gel electrophoresis. Conditioned medium and whole-cell extracts were sequentially passed through two Blue Sepharose columns. Fractions were collected, dialyzed, and tested for anti-growth activity. Human and hamster tumor cells grown as monolayers were treated with either (1) the purified fractions, (2) fractions pre-treated with trypsin (a protein specific enzyme), (3) fractions incubated at 75C, or (4) control medium. Fresh medium, crude conditioned medium, and crude whole-cell extract were used as controls. The results from these studies show that the growth inhibitory activity of the factor(s) was enhanced considerably by the purification procedure. Moreover, both the trypsin and heat-treatment destroyed the activity, indicating that the factor(s) is probably protein. Polyacrylamide gel electrophoretic studies show the presence of a protein band in fractions from the conditioned medium which co-migrates with human fibroblast interferon. A comparable band was not apparent in electrophoretic profiles from the whole-cell extract suggesting that the factor is distinct. (no Refs)

17. GROWTH OF HUMAN ADENOCARCINOMA OF THE LUNG (ACL) CELL LINES AND CLINICAL SPECIMENS IN SERUM-FREE DEFINED MEDIUM (MEETING ABSTRACT)

Brower M, Carney D, Ole H, Matthews M, Minna J

National Naval Medical Center, Bethesda, MD, 20014

Proc Am Soc Clin Oncol; 1:C-543 1982

A chemically defined medium containing insulin, transferrin, epidermal growth factor, selenium, hydrocortisone, and bovine serum albumin (LA medium) was tested for its ability to support the growth of 3 established ACL lines, NCI-H23, NCI-H125, and A549/8, and fresh specimens of ACL. Growth in the defined medium was compared to growth in serum supplemented medium (SSM). Successful growth of each cell line was observed in LA, and the doubling time of the cell lines in this medium was comparable to that observed in SSM. Of 6 fresh ACL specimens tested in LA medium and SSM, successful tumor growth was observed in 3/6 specimens in LA, all of which are still growing. Tumor growth was seen in only 1/6 specimens in SSM and this clinical specimen was overgrown with fibroblasts and tumor cells lost. No overgrowth of non-malignant stromal cells was observed in specimens in LA medium. The tumor cell origin of the cells cultured in LA medium was confirmed by cytology and flow cytometric examination. The selectivity of LA medium was confirmed by the failure of cytologically negative specimens to show growth in LA. (1 Ref)

18. TRANSFORMATION INDUCED BY ABELSON MURINE LEUKEMIA VIRUS INVOLVES PRODUCTION OF A POLYPEPTIDE GROWTH FACTOR.

Twardzik DR, Todaro GJ, Marquardt H, Reynolds FH, Stephenson JR

Lab. Viral Carcinogenesis, NCI, Frederick, MD, 21701

Science; 216(4548):894-897 1982

Rat embryo fibroblast transformed by Abelson murine leukemia virus (MuLV) produce and release a transforming growth factor (TGF). Production of this factor is correlated with a tyrosine-specific protein kinase that is functionally active and is associated with the major Abelson MuLV gene product, P120. Transformation-defective mutants of Abelson MuLV do not transform cells, do not have their virus coded transforming gene product phosphorylated in tyrosine, and do not induce TGF production. Abelson MuLV-induced TGF morphologically transforms cells in culture, competes with 125I-labeled epidermal growth factor (EGF) for binding to cell receptors, and induces phosphorylation of tyrosine acceptor sites in the 160,000-dalton EGF membrane receptor. After purification to homogeneity, Abelson virus-induced TGF migrates as a single polypeptide with an apparent size of 7,400 daltons as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. (Author abstract) (27 Refs)

19. HUMAN TRANSFORMING GROWTH FACTOR. PRODUCTION BY A MELANOMA CELL LINE, PURIFICATION, AND INITIAL CHARACTERIZATION.

Marquardt H, Todaro GJ

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J Biol Chem; 257(9):5220-5225 1982

A low mol wt human transforming growth factor (hTGFs) was isolated from serum-free medium conditioned by a human metastatic melanoma tumor line, A2058. The purification of hTGFs was achieved by gel permeation chromatography of

the acid-soluble growth-promoting activity on Bio-Gel P-10 in 1 M acetic acid, followed by reverse phase high pressure liquid chromatography on muBondapak C18 support using a linear gradient of acetonitrile in 0.045% trifluoroacetic acid, and subsequently by rechromatography of the human transforming growth factor-containing fractions with a linear 1-propanol gradient in 0.035% trifluoroacetic acid on the same column. The estimated mol wt of hTGFs is 7,400. It is a single chain polypeptide with three intrachain disulfide bridges and no free sulfhydryl groups. Lacking tyrosine and methionine, but containing three phenylalanine residues, hTGFs is unlike human and mouse epidermal growth factor (EGF). hTGFs competes with 125I-labeled EGF for binding to A431 human carcinoma cells completely and equivalently, and thus is functionally related to EGF. In contrast, hTGFs enabled normal anchorage-dependent rat kidney cells to grow in soft agar, whereas EGF did not stimulate growth of these cells in semisolid medium. The half-maximal growth-stimulating response of htGFs was reached with one EGF-competing unit or 1.1 ng of hTGFs. (Author abstract) (24 Refs)

20. AFFINITY LABELING OF THE PROTEIN KINASE ASSOCIATED WITH THE EPIDERMAL GROWTH FACTOR RECEPTOR IN MEMBRANE VESICLES FROM A431 CELLS.

Buhrow SA, Cohen S, Staros JV
(c/o Staros) Dept. Biochemistry, Vanderbilt Univ. Sch. Medicine, Nashville, TN, 37232
J Biol Chem; 257(8):4019-4022 1982

Epidermal growth factor (EGF), a mitogenic polypeptide hormone, stimulates the phosphorylation of certain endogenous proteins in membrane preparations derived from A431 cells, a human tumor cell line. Membrane vesicles prepared from A431 cells were reacted with 5'-p-fluorosulfonylbenzoyl adenosine (5'-p-FSO2BzAdo). Reaction of the vesicles with 5'-p-FSO2BzAdo results in a time-dependent inhibition of EGF-stimulable protein kinase activity which parallels an increase in incorporation into the vesicles of the 5'-p-sulfonylbenzoyl-[8-14]adenosine moiety from 5'-p-FSO2Bz[14C]Ado. The primary bands labeled have Mr = 170,000 and 150,000. Labeling of these bands by 5'-p-FSO2Bz[14C]Ado is inhibited by incubation of the membrane vesicles with adenyl-5'-yl imidodiphosphate, an ATP analog. Inactivation of the kinase with N-ethylmaleimide or by heating results in a sharply decreased labeling of the proteins with Mr = 170,000 and 150,000. Proteins of these mol wt have previously been identified in these cells as the EGF receptor and a degradation product of the receptor. These experiments provide chemical evidence that the EGF receptor and the EGF-stimulable kinase are the same protein. (Author abstract) (36 Refs)

21. TUMOR DEVELOPMENT IN THE HUMAN COLON IS ENHANCED BY EPIDERMAL GROWTH FACTOR (UROGASTRONE), DEOXYCHOLIC ACID, AND AN ENDOGENOUS ANALOG TO TPA (MEETING ABSTRACT)

Friedman EA
Memorial Sloan-Kettering Cancer Center, New York, NY
Conference on Cell Proliferation, Cancer and Cancer Therapy, February 17-19, 1982, New York, New York New York Academy of Sciences; :AP-5 1982

Many human neoplasms arise through a series of progressive cellular changes. Premalignant cells in the colon form a benign tumor or adenoma, from which a carcinoma develops. Benign tumors fall into three classes based on their pathology and clinical prognosis. Premalignant epithelial cells cultured from each class reflect the tumor's position in the progression to carcinoma. Cells from early-stage (tubular) adenomas are stimulated to divide by EGF(epidermal growth factor) (structurally similar to urogastrone, a tropic hormone in the intestine), by TPA (12-O-tetradecanoylphorbol-13-acetate), and by deoxycholic acid, a secondary bile acid found in the colon. Cells from either intermediate-stage (villotubular) or late-stage (villous) adenomas are not stimulated to divide by EGF or by deoxycholic acid. TPA-treated intermediate and late-stage premalignant cells from rills and clusters several cells in thickness and secrete a plasminogen activator. This multilayering is reminiscent of areas of carcinoma found within adenomas. Furthermore, it suggests that a endogenous compound which binds to TPA receptors on late-stage premalignant cells, and so mimics TPA activity, is important in the transition to malignancy. (no Refs)

22. NEUROFIBROMATOSIS FIBROBLASTS: ABNORMAL GROWTH AND BINDING TO EPIDERMAL GROWTH FACTOR.

Zelkowitz M
Dept. Pediatrics, Univ. Chicago, Chicago, IL, 60637
Adv Neurol; 29:173-189 1981

Neurofibromatosis (NF) skin fibroblasts [especially after exposure to epidermal growth factor (EGF)] were examined to test the hypothesis that NF is a disorder of growth and proliferation. Results involving nine sets of age- and passage-matched NF and normal fibroblast strains suggest that NF skin fibroblasts have a cytoplasmic membrane defect related to a diminished ability to bind and respond to EGF, suggesting a possible explanation for the abnormal growth phenomena in NF. (42 Refs)

23. EPIDERMAL GROWTH FACTOR INDUCES RAPID CENTROSOMAL SEPARATION IN HELA AND 3T3 CELLS.

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Dept. Medicine, Div. Endocrinology, Univ. Connecticut Health Center, Farmington, CT, 06032
J Cell Biol; 93(2):507-512 1982

Using indirect immunofluorescence, we have found that epidermal growth factor (EGF), at 100 ng/ml, induces centrosomal separation within 20 min in HeLa and 3T3 cells. The effect was evident both in unsynchronized cultures and in HeLa cells blocked in early S phase by hydroxyurea. EGF also induced centrosomal separation in quiescent 3T3 cells blocked in G0/G1 by serum deprivation, indicating that DNA replication is not necessary for this effect. The mechanism of this rapid centrosomal separation and its role in the mitogenic effects of EGF remains to be determined. (Author abstract) (45 Refs)

24. REGULATION OF INTRACELLULAR PROTEIN DEGRADATION BY INSULIN AND GROWTH FACTOR.

Ballard FJ, Niehl MK, Francis GL, Knowles SE
CSIRO Div. Human Nutrition, Kintore Ave., Adelaide S. A. 5000, Australia
Acta Biol Med Ger; 40(10/11):1293-1300 1981

Some of the properties of growth factor (GF) action on protein breakdown (PBD) in cultured cells are described. Insulin, epidermal GF (EGF), fibroblast GF (FGF), insulin-like GF 1 (IGF-1), insulin-like GF 2 (IGF-2), and fetal calf serum (FCS) were tested for inhibitory effects on PBD in 19 cell lines. Ex-

amples of cells which are sensitive to other GF are presented. PBD in all cells tested was inhibited by FCS, IGF-1, and IGF-2. GF produce a stimulation of DNA synthesis. The addition of IGF-1 to HTC hepatoma cells showed that withdrawal of GF is accompanied by a rapid change to the higher rate of PBD. Marked inhibitions of PBD were observed in HTC hepatoma cells with insulin, IGF-1, and FCS but when these GF are added to the cells at concentrations known to give maximal effects, no additivity was observed. Concentration effects of GF in IMR90 fibroblasts, HTC, and MH1C1 hepatoma cells are presented. When effects of both insulin and IGF are considered together, PBD is reduced selectively in transformed cells. PBD is much more effectively inhibited by insulin, EGF, IGF-1, IGF-2, or FCS in the mid-passage than in the senescent cells. The poorer response does not appear to reflect an already low rate of autophagic proteolysis in the aged cells because inhibition of lysosomal proteolysis by ammonia gives an equal response in both mid-passage and aged cells. PBD is inhibited by 0.5% colostrum to approx the same extent as found with 10% FCS in six lines and only slighty less in MCF7 and OS104 cells. In both the H35 hepatoma and Swiss 3T3 mouse fibroblasts, 0.5% colostrum is more effective than 10% FCS. (9 Refs)

25. DNA SYNTHESIS IN DIFFERENTIATING NEUROBLASTOMA CELLS FOLLOWING INDUCTION BY GLIOMA CELLS.

Allin EP, Wittendorp-Rechenmann E, Winsor B, Rechenmann RV
Laboratoire de Physiologie et de Biologie Cellulaire, Universite Louis Pasteur, Strasbourg, France
Cell Mol Biol; 28(1):37-41 1982

Transient co-culture of neuroblastoma cells with glioma cells has been used to induce differentiation in the neuroblastoma. The rate of DNA synthesis has been measured for 6 days following induction of differentiation. The initial rate of synthesis in differentiating cells is less than half that of the controls and the rate is maintained over the 6 days while that of the control cells falls to a low level from the 3rd day of culture. The distribution of label in induced and in non-induced cells has been followed by autoradiography. On the 6th day of culture 70% of the induced cells appear labeled while 40% of the non-induced cells show labeled nuclei. The induced cells are morphologically differentiated while the non-induced cells show few axon-like processes. (Author abstract) (17 Refs)

26. REGULATION OF MUSCARINIC LIGAND BINDING SITES BY NERVE GROWTH FACTOR IN PC12 PHAEOCHROMOCYTOMA CELLS.

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Nature; 297(5862):152-154 1982

Rat pheochromocytoma PC12 cells contain saturable, high-affinity binding sites for quinuclidinyl benzilate (QNB). Scatchard analysis of this binding resembles that of QNB binding to muscarinic cholinergic receptors of rat brain, smooth muscle and adrenal medulla. Treatment with nerve growth factor produces a marked increase in QNB binding. PC12 cells may thus provide a model system for studying the biochemistry of muscarinic function and the effect of hormones and trophic substances on the function and expression of these receptors. (24 Refs)

27. INDUCTION OF NEUROFILAMENT TRIPLET PROTEINS IN PC12 CELLS BY NERVE GROWTH FACTOR.

Lee V, Trojanowski JQ, Schlaepfer WW
Div. Neuropathology, Dept. Pathology, Univ. Pennsylvania Medical Sch., Lab. Medicine, Philadelphia, PA, 19104
Brain Res; 238(1):169-180 1982

Indirect immunofluorescence and monospecific, immunosorbent purified antibodies to 68,000, 150,000, and 200,000 dalton neurofilament proteins were used to localize neurofilament triplet proteins in PC12 cells grown in the absence of (PC12-) or in the presence of (PC12+) nerve growth factor (NGF). PC12-cells, a neoplastic counterpart of adrenal chromaffin cells, which resemble undifferentiated primitive sympathetic or 'blast' cells, had low levels of neurofilament proteins. Subsequent to being exposed to NGF, they became PC12+ cells and developed features observed in postmitotic sympathetic neurons. This transition from a pre- to postmitotic state coupled with neuronal differentiation also coincides with the induction of high levels of neurofilament proteins. Thus the induction of neurofilament proteins is another marker of neuronal differentiation in this cell line. However, the synthesis and induction of neurofilament proteins in PC12 cells deviated from that which occurs in the normal counterpart of PC12+ cells, ie, sympathetic neurons, since in PC12 cells they apparently do not assemble into intact neurofilaments. Failure of neurofilament proteins to assemble into intact neurofilaments indicates a complexity in neurofilament protein synthesis and assembly which has not been previously recognized or documented. It further indicates that the PC12 cell line is not only a useful model for studies of neuronal differentiation but also for studies of neurofilament synthesis, assembly, and ontogeny. (24 Refs)

28. NEURAL CREST DIFFERENTIATION AND RESPONSE TO NERVE GROWTH FACTOR IN MURINE EMBRYOS.

Kessler JA, Cochard P, Black IB, Weston, Bolande, Riccardi, Riopelle
Lab. Developmental Neurology, Dept. Neurology, Cornell Univ. Medical Coll., New York, NY, 10021
Adv Neurol; 29:115-123 1981

Studies of the effects of the influence of exogenous neuronal growth factor (NGF) on rat embryos and on mouse sympathetic ganglia are reviewed. Temporal aberration of transmission or reception of appropriate signals needed for development may result in developmental abnormalities that lead to the presence (or absence) of normal or abnormal cellular populations. (24 Refs)

29. RECEPTOR INTERACTIONS OF NERVE GROWTH FACTOR.

Riopelle RJ, Sutter AK, Harris-Warrick RM, Klearman M, Shooter EM, Hall, Weston
Dept. Medicine (Neurology), Queen's Univ., Kingston, K7L 3J7 Ontario, Canada
Adv Neurol; 29:133-148 1981

The chemical characteristics of the components of receptors for nerve growth factor (NGF) found on dorsal root ganglia and sympathetic cells, and binding heterogeneity, are discussed. Results for kinetics of binding of beta-NGF to day 8 chick dorsal root ganglion membrane preparations are discussed in relation to observed nonlinearity of Scatchard plots. The number and roles of binding sites and the possibility that NGF is processed following binding are considered. (30 Refs)

30. INTERACTIONS BETWEEN SCHWANN CELLS (SCS) AND NERVE GROWTH FACTOR (NGF)-RESPONSIVE PC12 PHEOCHROMOCYTOMA CELLS IN CULTURE (MEETING ABSTRACT)

Cochran M, Black MM
Department of Anatomy, Temple University Medical School, Philadelphia, PA
Anat Rec; 202(3):33A 1982

Purified mouse SCs and NGF-pretreated PC12 cells were co-cultured for up to 49d to determine whether 1) PC12 cell growth upon a SC bed would lead to neurite regrowth and maintenance in the absence of exogenously added NGF, and 2) SCs would recognize and ensheath PC12 neurites. Purified beds of quiescent SCs (without neurons) were prepared from C57bl mouse dorsal root ganglia (DRGs) by methods modified from Wood. SCs were maintained for 1 wk in NGF free medium perior to cell recombination. In the absence of SCs, regrowth and maintenance of neurites by NGF pretreated PC12 cells requires exogenous NGF. In combined cultures of SCs and PC12 cells grown with NGF for 19d, most or all PC12 cells had neurites regardless of whether they were on or off the SC bed. In combined cultures grown without NGF for 19d, 66% of PC12 cells or cell clusters located on SC beds had neurites, while 9% of PC12 cells not on SC beds had neurites. These observations suggest that 1) SCs can replace exogenously added NGF in promoting regrowth and maintenance of neurites by NGF-pretreated PC12 cells, and 2) neurite regrowth and maintenance in combined cultures without NGF depends on contact between SCs and PC12 cells. Electron micrographs of SC-PC12 cell areas in + NGF and -NGF cultures at 19 and 34d showed that most PC12 cell neurites were situated in shallow grooves of the SC surface, but that relatively few neurites were completely ensheathed by SCs. SCs associated with PC12 cell neurites were not covered by a basal lamina. (1 Ref)

31. THE INDUCTION OF DIFFERENTIATED FUNCTIONS IN HUMAN LEUKEMIC CELL LINES (MEETING ABSTRACT)

Brewer G, Briggs RC, Hallick LS, Bloch D, McArthur B
Department of Biochemistry, Vanderbilt University School of Medicine, Nashville, TN, 37232
Med Pediatr Oncol; 10(1):101 1982

Two human myeloid leukemic cell lines HL-60 and KG-1 and the undifferentiated blast cell line K-562 all respond in varying ways to inducers of cell differentiation. Recently, it was discovered that addition of hemin to cultures of K562 induces synthesis of fetal and embryonic globins. During five days of hemin treatment, globin was detected after the first days and was maximal at five days. DNase I, which is known to preferentially digest active genes, was used to digest nuclei isolated from cultures of K562 cells either untreated or containing 30uM hemin for one day. Proteins from the soluble fraction were obtained from the nuclei by centrifugation and subjected to SDS (sodium dodecyl sulfate) polyacrylamide gel electrophoresis. Addition of hemin to cultures of HL-60 cells did not result in the induction of globin and is used as a control. The human promyelocytic leukemic cell line HL-60 was induced by retinoic acid to differentiate. Retinoic acid did not induce differentiation in KG-1 or K562 cells. Antisera have been raised to dehistonized chromatin preparations from each of the untreated cultures of these cell lines. Changes in the nonhistone proteins were detected in the treated cells by using the peroxidase immunochemical detection of antigens in chromosomal proteins transferred from SDS polyacrylamide gels to sheets of nitrocellulose. Pertubations in the cell cycle were observed in the retinoic acid treated HL-60 cells by using flow cytometric analysis of acriflavin-Feulgen stained DNA and light scatter. These approaches provide a very sensitive means for analyzing the effects of inducers on these cells. (no Refs)

32. GROWTH FACTORS IN HODGKIN'S DISEASE.

Ford RJ, Mehta S, Davis F, Maizel AL
Dept. Anatomic Pathology, Univ. Texas System Cancer Center, M.D. Anderson Hosp. and Tumor Inst., 6723 Bertner Ave., Houston, TX, 77025
Cancer Treat Rep; 66(4):633-638 1982

Tumor nodules from spleens removed at staging laparotomy for nodular sclerosing Hodgkin's disease were grown as fragment cultures in vitro. These cultures could be maintained in vitro for up to 2 months and consisted of 90% large, adherent multinuclear and mononuclear cells. Immunologic markers and enzyme histochemistry suggested that the cultured cells belonged to the monocyte/macrophage lineage. Supernatants from these cell cultures were then assayed for the monokine Interleukin 1 and for fibroblast stimulatory factors, which were both present. These results are discussed in considering Hodgkin's disease as a neoplasm of the monocyte/macrophage lineage, capable of producing functionally active mediators that may account for the histopathologic appearance observed. (Author abstract) (20 Refs)

33. INHIBITION OF THYMIDINE UPTAKE OF TUMOR CELLS BY PLATELET LYSATE (MEETING ABSTRACT)

Landolfi R, Steiner M
Division of Hematologic Research, The Memorial Hospital, Pawtucket, RI, 02860
Fed Proc; 41(3):334 1982

Platelets are known to contain growth factors for normal and tumor cells. We have studied the effect of human platelet lysate (HPL) on the incorporation of [3H]-thymidine by mouse neuroblastoma (Neuro-2a) cells. Actively growing Neuro-2a cells were pulse labeled with [3H]-thymidine at various concentrations (0.012-0.05 nmol/ml). Thymidine incorporation into DNA was measured by preparing a perchloric acid extract of the tumor cells. Addition of HPL produced a maximal reduction of thymidine incorporation ranging from 38-51% which was independent of the thymidine concentration used. A linear dose dependency of the suppressant effect of HPL was demonstrated up to about 100 ug protein/ml. In other experiments Neuro-2a cells, synchronized in G1 by serum deprivation, were continuously exposed to low concentration of [3H]-thymidine (2.5 pmol/ml) for up to 48 hours. HPL inhibited [3H]-thymidine incorporation by 81-88% compared to controls. The presence of fetal calf serum up to 10% was unable to overcome the inhibition exerted by HPL. Removal of HPL from the medium completely restored normal thymidine uptake. The inhibitor was found to be non-dialyzable, heat sensitive (75C for 30 min) and acid resistant (pH 4.5). A MW of approximately 100,000 was estimated by gel filtration. These findings show that platelets contain both positive and negative modulators of tumor cell growth. (no Refs)

34. INDUCTION OF DIFFERENTIATION OF HL-60 LEUKEMIC CELLS BY POST-ENDOTOXIN SERUM (MEETING ABSTRACT)

Chiao JW, Bressler J, Pinsky C, Hirshaut Y, Oettgen HF, Clarkson B
Sloan-Kettering Institute for Cancer Research, New York, NY, 10021
Proc Am Assoc Cancer Res; 23:893 1982

Serum from cancer patients treated with endotoxin was tested for activity for induction of differentiation of leukemic cells in vitro. Liquid cultures of HL-60 human leukemic promyelocytes were supplemented with 20% pre- or post-endotoxin serum from patients with melanoma or ovarian carcinoma injected with endotoxin. Cultures supplemented with post-endotoxin sera showed a significant increase in the number of cells with membrane complement receptors, markers for differentiation. This did not occur in control cultures incubated with pre-injection serum from the same individual. Approximately 7.5% of the cells cultured with post-endotoxins sera displayed complement receptors after three days, compared to none in the initial cultures and in three day control cultures. The proportion of cells with complement receptors reached a peak on day five with a mean of 12.5% and a range of 5-29%. The increase in the absolute number of cells with complement receptors was much higher than the increase in total cell number which suggests that the presence of these cells was due to differentiation rather than selective proliferation or cell death. Induction of differentiation was mediated by serum collected 2-12 hours after injection of endotoxin. Incorporation of 3H-thymidine into replicating cells was tested as a measure of proliferation of HL-60 cells during induced differentiation. A decrease in proliferation was evident in cultures supplemented with post-endotoxin serum. This decrease always preceded the appearance of complement receptor bearing cells. (no Refs)

35. DIFFERENTIATION OF A HUMAN HISTIOCYTIC LYMPHOMA CELL LINE (U937) BY A PHOSPHOLIPASE A2 INHIBITORY PROTEIN (LIPOMODULIN) OR GLUCOCORTICOIDS (MEETING ABSTRACT)

Hattori T, Hoffman T, De Bles A, Hirata F
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Fed Proc; 41(3):793 1982

Human and mouse myeloid leukemia cell lines differentiate when cultured with glucocorticoids. Glucocorticoids may act in part via induction of lipomodulin, a phospholipase A2 inhibitory protein. A human histiocytic lymphoma cell line (U937) with characteristics of monocytes was employed to study the effects of glucocorticoids and lipomodulin on cellular differentiation. After 3 days of culture with lipomodulin (5 x 10-9M), cell growth was inhibited 50-80%. Cells became adherent, and demonstrated increased cell size, number of azurophilic granules, and membrane ruffling. The treated cells also began to express a myelomonocytic antigen defined by a monoclonal antibody (B43.4.1). These cells were active in antibody-dependent cellular cytotoxicity against chicken red blood cells. Each of these effects was inhibited in a dose-dependent manner by pretreatment of lipomodulin with anti-lipomodulin monoclonal antibody. Differentiation induced by glucocorticoids (10-6M dexamethasone) was manifested by changes similar to those induced by lipomodulin, but seemed to occur later. The actions of glucocorticoids which result in cell differentiation might be mediated through lipomodulin, in a manner similar to their anti-inflammatory effects. (no Refs)

36. GROWTH REGULATORY FACTORS IN CONDITIONED MEDIA FROM S49 LYMPHOMA CELLS GROWN UNDER SERUM-FREE CONDITIONS (MEETING ABSTRACT)

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Fed Proc; 41(3):495 1982

A serum-free medium has been devised which contains casein, insulin, transferrin, testosterone, and linoleic acid (CITTL). This medium supports the growth of normal and malignant lymphoid cells. Murine S49 lymphoma cells grown in CITTL for 3 months have doubling times indistinguishable from those grown in serum-containing media. The use of conditioned media from cells grown without serum is advantageous for the isolation of secretory factors from cultured cells. In this regard, conditioned medium from 349 cells grown in CITTL to log phase was observed to contain a factor which promotes the clonal growth of these cells. This factor is precipitated by ammonium sulfate, is nondialyzable (greater than 1000 daltons) but passes through an Amicon PM10 filter. The activity of this 1000-10,000 dalton fraction is concentration dependent. In addition, a second factor, which is growth inhibitory has been identified in conditioned medium. This factor is found in the 500-1000 dalton fraction from S49 cells grown to a cell density of 1.2-1.5x10(6) cells/ml. Cells growing at log phase arrest in the G1 phase of the cell cycle in the presence of this factor. I propose that these factors are secretory products of S49 cells which autoregulate the growth of these lymphoma cells. (no Refs)

37. INDUCTION OF DIFFERENTIATION IN MURINE B CELL LYMPHOMAS (MEETING ABSTRACT)

Lanier LL, Warner NL
Department of Pathology, University of New Mexico School of Medicine, Albuquerque, NM, 87131
Fed Proc; 41(3):425 1982

Previous studies have demonstrated that murine B lymphoma cell lines are responsive to biological signals. However, in most cases, these studies have been limited in scope, often only determining the ability of a single cell line to proliferate or synthesize Ig. In contrast, we have examined the ability of a variety of biological agents to induce differentiative changes in an extensive panel of B lymphoma cell lines, which are apparently arrested at different points in maturation. We have examined the phenotypic, proliferative, and functional changes accompanying activation, and changes in surface density of a panel of differentiation antigens defined by monoclonal antibodies using quantitative flow cytometry analysis. For example, lipopolysaccharide (LPS) induced a 3-4 fold increase in surface IgM and the expression of the ThB antigen on the NBL line, but a decrease in surface IgG and Ia antigens on the 2PK3 line. In contrast, LPS activation of L10A/2J resulted in an increase in I-A and I-E/C gene products, without influencing surface expression of H-2D and H-2K antigens. These changes occurred without significant influence on cell proliferation or DNA profile (cell cycle). Studies are underway to examined the influence of soluble T cell factors on these B cell lines. (no Refs)

38. SOLUBLE GROWTH FACTORS (NON-INTERLEUKIN-2) (IL-2) PRODUCED BY MALIGNANT LYMPHOID CELLS (MEETING ABSTRACT)

Ulttenbogaart C, Fahey JL
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Proc Am Assoc Cancer Res; 23:1032 1982

In order to identify new growth factors (GF), produced by malignant T lymphoid cell lines, MOLT-4f and HSB-2 were cultured long-term in serum free (SF) medium, which consisted of Iscove's modified Eagle's medium, supplemented with bovine serum albumin and transferrin. Supernatants (SN) were collected 2x/week and pooled before purification. Partial purification was obtained with ammonium sulfate precipitation, followed by AcA 54 gel diffusion chromatography. Crude SN's and fractions were tested for growth promoting activity on MOLT-4f and HSB-2 cells and on an IL-2 dependent murine cytotoxic T cell line (CTLL-2). MOLT-4f and HSB-2 SN's contained a GF(s) for both cell lines, but did not stimulate growth of non-T cells and did not contain IL-2 activity. The growth factor(s) were present in the fractions with a molecular weight smaller than IL-2. The soluble growth factor(s) described here may provide a basis for the ability of the leukemic cell lines MOLT-4f and HSB-2 to proliferate independently in culture. (no Refs)

39. GROWTH INHIBITION (GI) OF UNDIFFERENTIATED LYMPHOMA (UL) CELLS BY SURFACE IGM MODULATION (MEETING ABSTRACT)

Parsons RG, Magrath IT
National Cancer Institute, Frederick Cancer Research Facility, Frederick, MD, 21701
Proc Am Assoc Cancer Res; 23:1005 1982

We have initiated a series of studies to determine the effects of surface IgM modulation on the growth of human UL cell lines. IgM modulation was affected with low doses (7ug/ml) of anti-IgM (u specific). The majority (5/7) of the American UL cell lines showed major GI (53-94%) as a result of surface IgM modulation. GI (greater than 30%) was not detected in any African Burkitt's lymphoma cell lines (3), Epstein Barr Virus (EBV)-transformed cord blood lymphocyte lines (3), or other EBV-containing lymphoblastoid cell lines (2). American UL cell lines inhibited by anti-Igm treatment did not contain the EBV genome, whereas lines which displayed weak GI were EBV+. GI of the American UL cell lines was observed following treatment with antibodies directed against u-heavy chain or the specific light chain class (kappa) that each cell line displayed, however GI was not detected following exposure of the cells to F(ab)'2 fragments directed against IgM heavy chain or antisera against other cell surface determinants. GI occurred rapidly with detectable effects at 2-6 hours after exposure to antibodies and maximum inhibition of (3H)-thymidine incorporation at 24 to 48 hours after treatment. GI was not associated with marked cytotoxicity. We have previously hypothesized that the American UL cells represent immature B-lymphocytes. This GI effect may be related to the induction of tolerance in neonates by anti-IgM antisera. (no Refs)

40. DOES AN AUTOLOGOUS MIXED LYMPHOCYTE REACTION ACCOUNT FOR THE 'SPONTANEOUS' PROLIFERATIVE ACTIVITY OF PERIPHERAL BLOOD MONONUCLEAR CELLS?

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Cell Immunol; 68(1):173-180 1982

Background and 'spontaneous' proliferation are terms often used for the proliferative activity normally exhibited by peripheral blood mononuclear cells (MNC) in vitro. In this report, we show that Interleukin-2 (IL-2) added to unfractionated MNC but not to isolated T or non-T cells significantly increased their proliferative activity. The cells responding to IL-2 stimulation from MNC were OKT3 positive lymphocytes. In addition, treatment of MNC with either a monoclonal anti-HLA-DR antibody (in the absence of C') or Cyclosporin-A strongly suppressed the 'background' whereas treatment of MNC with the 3A1 monoclonal anti-human T cell antibody did not modify 'spontaneous' proliferation of these cells. IL-2 could not restore or increase the proliferative activity of MNC exposed to the anti-HLA-DR antibody or Cyclosporin-A while the T cell growth factor significantly enhanced proliferation of MNC cultured in the presence of the OKT4 antibody. Taken together these results strongly suggest that IL-2 responding T cells from MNC become sensitive to IL-2 by interacting with HLA-DR antigens on B lymphocytes and/or monocytes contained in MNC (resting T cells are Dr-). By a similar mechanism we have previously shown that T cells acquire responsiveness to IL-2 in the autologous mixed lymphocyte reaction (AMLR). Since all the cells that participate in AMLR are present in MNC, we postulate that a 'mini' AMLR taking place within MNC may explain the 'spontaneous' proliferation of peripheral blood mononuclear cells. (Author abstract) (12 Refs)

41. HUMAN LYSOZYME INDUCES PROLIFERATION OF HUMAN PERIPHERAL BLOOD LYMPHOCYTES (MEETING ABSTRACT)

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Fed Proc; 41(3):608 1982

Lysozyme (LZM) is a major product of monocytes and macrophages. Human LZM was isolated from the urine of patients with monocytic leukemia by absorption to bentonite and elution with pyridine:sulfuric acid followed by ion exhange chromatography on carboxymethyl cellulose. Peripheral blood mononuclear leukocytes (PBML) depleted of cells adherent to plastic were cultured in a standard medium with 10% autologous serum at a cell concentration 1 x 10(6)/ml for six days on microtiter plates. Human LZM at concentration of 4 to 63 ug/ml induced blast transformation and a 3 to 30-fold increase in 3H-thymidine uptake of the PBML of all subjects (9 male: 9 female, age 29-67 yrs). A similar response was obtained with 2 hr pretreatment of PBML with human LZM prior to culture without LZM. The transformed blasts were E (erythrocyte) rosette positive (E+) and stained with monoclonal antisera to T cells (OKT3 and OKT4/OKT8). The LZM effect was inhibited by rabbit antiserum to human LZM. Separated E+ or E- cells did not respond to human LZM. Pretreatment of cultures with anti-Dr + complement abrogated the LZM effect. No PBML proliferation was seen in the presence of hen egg white LZM or a human LZM preparation where the active site was blocked by 2',3'-epoxypropyl beta-glycoside of di-N-acetyl-D-

glucosamine. The results demonstrate that human LZM stimulates lymphocytes probably through its enzymatic activity. (no Refs)

42. THE ALPHA CHAIN, NOT THE BETA CHAIN OF HLA-DR ANTIGENS PARTICIPATES IN ACTIVATION OF T CELLS IN AUTOLOGOUS MIXED LYMPHOCYTE REACTION.

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Immunogenetics; 15(4):341-356 1982

Using antisera against the alpha, the beta or the complex of both chains of HLA-DR antigens, we have studied the role of individual chains of HLA-DR antigens in activation of T cells in autologous mixed lymphocyte reaction (AMLR). Alpha chain-specific antibody, not anti-beta chain serum prevented T cells from acquiring responsiveness to interleukin-2 (IL-2), suppressed the production of IL-2, and inhibited the T cell proliferative response in both primary and secondary AMLR cultures. However, proliferation of already activated IL-2 reactive T cells supported by IL-2 was not affected by any of the four different types of anti-DR sera used. Fifty to sixty percent of T cells activated by AMLR or by PHA possessed DR antigens and functioned well as stimulator cells in secondary AMLR cultures. Moreover, the stimulatory activity of these DR-positive T cells was suppressed by the anti-alpha chain, not by the beta chain-specific antibody. Since continuous proliferation of T cells requires IL-2 and since nonactivated T cells are not sensitive to IL-2 and are unable to absorb this growth factor, we conclude the following: (1) The alpha, not the beta chain of HLA-DR antigens seens to be the structure responsible for enabling resting T cells to respond to IL-2 and induce production of IL-2 in AMLR. (2) Once T cells have acquired responsiveness of IL-2 and the growth factor has been produced, there is no further requirement for HLA-DR antigens, but the availability of IL-2 determines the level and extent of proliferation of IL-2 sensitive T cells. (Author abstract) (45 Refs)

43. USE OF MICE THYMOCYTES FOR TESTING OF BIOLOGICAL ACTIVITY OF 'MEDIUM-SIZE MOLECULES' IN THE BLOOD PLASMA OF CANCER PATIENTS.

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Eksp Onkol; 4(2):67-69 1982

The cytotoxic activity of the fraction of 'medium-size molecules' (MSM) from the blood plasma of cancer patients was studied in the thymocytes of BALB/c mice (target cells). Incubation of MSM fraction from 10 cancer patients (5 had Stage IV lung cancer, 4 had Stage IV stomach cancer, and 1 had chronic lymphoid leukemia) with the test target cells showed that cytotoxicity depended upon the concentration of polyamine and peptide components in the MSM. (14 Refs) (In RUS)

44. AN ACUTE LYMPHOBLASTIC LEUKEMIA WHICH PRODUCES HUMAN T CELL GROWTH FACTOR (MEETING ABSTRACT)

Anderson DW, Hayward A, Jones C
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Fed Proc; 41(3):494 1982

T cell growth factor (TCGF) or interleukin-2 is a lymphokine that supports the long-term replication of activated T cells. The production of TCGF by human acute lymphoblastic leukemia cell lines was studied. We report that stimulation of the HSB-2 line (10(6) cells/ml) by PHA (phytohemagglutinin) (1% PHA-M; Difco) gave a supernatant which contained TCGF activity greater than that obtained from tonsilar or peripheral blood lymphocytes. Factor production was enhanced by the addition of phorbol myristate acetate (20 ng/ml) to the cultures. The growth factor will enhance the proliferation of PHA stimulated thymocytes and sustain the proliferation of growth-factor dependent human PPD (purified protein derivative) specific T cell blasts. While these activities are characteristic of human TCGF, the HSB-2 cells lack the OKT3 and OKT4 surface markers previously associated with TCGF production. Furthermore, the TCGF made by HSB-2 cells eluted from Sephadex G-100 in the same position as the 45,000 MW marker. This cell line should be of great value in studies of the cell biology and molecular biology of factors which promote T cell growth. (no Refs)

45. ACTIVITY OF HUMAN LEUKOCYTE INTERFERON IN A HUMAN TUMOR CLONING SYSTEM.

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Cancer Chemother Pharmacol; 8(1):99-103 1982

The human tumor cloning system of Hamburger and Salmon was used to investigate the antitumor effect of human leukocyte interferon (IF) in material from 29 bone marrow aspirations, 53 tumor biopsies, 14 paracenteses, and 10 thoracenteses in 106 patients with advanced malignancies. Colonies in culture were formed in 62/106 attempts. IF caused a greater than or equal to 70% decrease in tumor colony forming units (TCFU; at a concentration of 1,000 U/ml) in only five cultures, one each of lymphosarcoma cell leukemia, small cell lung cancer, adenocarcinoma of the lung, breast cancer, and pancreatic cancer. IF caused an increase in the number of colonies over control growth in three cultures: one adenocarcinoma of the lung (82% increase), and two ovarian cancers (39% and 141% increase). Of the 62 dose-response studies conducted, IF had minimal or no effect on 15 different tumors in vitro, even at concentrations as high as 1,000 U/ml; only three patients (lymphosarcoma cell leukemia, small cell lung cancer, and pancreatic carcinoma) had greater than or equal to 70% decrease in TCFUs at the highest concentration of IF used (1,000 U/ml). The antitumor effect of IF was studied in one patient with lymphosarcoma cell leukemia with an 85% decrease in the number of colonies formed in vitro. Clinically, IF resulted in a decrease in the size of the lymph nodes, but circulating leukemic blast cells continued to be present. The cloning system may be useful for identifying patients who might experience an antitumor effect from IF. (26 Refs)

46. ANTIPROLIFERATIVE ACTIONS OF INTERFERON AND DOUBLE-STRANDED RNA: INDUCTION OF RESISTANT VARIANTS IN HUMAN TUMOR CELL POPULATIONS (MEETING ABSTRACT)

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Fed Proc; 41(3):750 1982

Human fibroblast interferon (IFN) and the dsRNA, rIn: rCn were found to exert a pronounced inhibitory effect on the proliferation of fibrosarcoma (HT1080) and bladder carcinoma (RT4) cells, completely arrested at 400 u/ml and 80 u/ml of IFN respectively. This inhibitory effect was much less on

normal human fibroblasts, only partially inhibited at 400-1000 u/ml IFN. Culture of HT1080 and RT4 cells in IFN-containing medium resulted in the emergence of subpopulations resistant (IFNr) to the antiproliferative effects of IFN. Luria-Delbruck fluctuation analysis indicated that the IFNr trait is nonrandomly acquired and is induced by IFN selection with a relatively high (0.1-4%) but varying frequencies within different IFN-sensitive (IFNs) clonal populations. Preliminary results indicate that the IFNr trait reverts to IFNs within 5-10 cell divisions when cultured in the absence of IFN. The IFNr cells retained sensitivity to the antiviral effects of IFN and were sensitive to the antiproliferative effects of rIn: rCn even in the presence of antibodies to IFN. For IFNr-RT4 cells, 2 of 4 clones acquired resistance to dsRNA toxicity at frequencies above parental cells. Since IFNr and dsRNAr traits can be acquired independently and transiently through a nonmutational mechanism, the antiproliferative actions of IFN and rIn:rCn may not be identical. (no Refs)

47. CHANGES IN DNA-POLYMERASE AND THYMIDINE KINASE ACTIVITY DURING INTERFERON TREATMENT.

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Biochem Biophys Res Commun; 105(4):1569-1576 1982

The effect of human interferon beta (IFN-beta) on the human glioma cell line, U-251 MG, was investigated. Exponentially growing cells treated with IFN exhibited a dose-dependent growth depression observed after 3 days, and an increase in the amount of cells in S phase. However, the activity of DNA polymerase was decreased to 30% of control levels at day 5 with 1,000 Units/ml of IFN-beta. This depression in enzyme activity may be explained by an inhibitory effect of IFN on the increase of enzyme activities induced by events in late G1 or early S, or by the induction of diffusable factors depressing enzyme activities. Starved cells were induced to cycle and DNA content was followed cytofluorometrically. S phase started approx 12 hr after addition of serum to synchronized cells and cells entered G2 at 21 hr. In contrast, IFN-treated cultures initiated S but accumulated in that phase. At the start of S phase, both DNA polymerase and thymidine kinase activity increased, although the rate of increase was markedly reduced by treatment with IFN. No inhibitory factor could be identified in the IFN treated cultures. It is probable that IFN has a direct effect on the induction of the studied enzyme activities, thus affecting virus replication. (18 Refs)

48. MODULATION OF HUMAN NATURAL KILLER CELLS BY INTERFERON AND PGE2 (MEETING ABSTRACT)

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9th International RES Congress, February 7-12, 1982, Davos, Switzerland. American Reticuloendothelial Society 68 pp., 1982.

It is becoming increasingly evident that natural killer (NK) cells may play an important role in immunosurveillance against cancer. Relatively little is known about the mechanisms involved in the regulation of endogenous or activated NK cells. The focus of this study is an examination of some of the mechanisms involved in the regulation of human NK cells. Stimulation of purified lymphocytes by interferon (IFN) (10(3) U/ml) or polyinosinic acid-polycytidylic acid (poly I:C) (100ug/ml) for 18 hr at 37 C results in augmented NK activity against K562 targets in a 2 hr 51Cr release assay. The presence of monocytes during the stimulation with poly I:C (18 hr) was shown to decrease the levels of augmentation and to depend on the concentration of monocytes. Supernates of highly purified monocytes (97-99% peroxidase+) cultured for 18 hr in the presence of poly I:C (100ug/ml) caused suppression of endogenous NK activity when added to an NK assay with nonadherent (NA) effector cells. These supernates contained significant amounts of PGE2 (prostaglandin E2) (2.5 to greater than 4.9x10-8M), but contained almost no anti-viral activity (less than 10 units/ml). In contrast, supernatants of poly I:C stimulated purified NA lymphocytes augmented the NK activity of NA cells. The augmentation correlated with IFN levels detected in those supernatants (100-1200 u/ml); they lacked, however, appreciable levels of PGE2. Interestingly, IFN or poly I:C activated NA cells or Percoll gradient enriched NK cells (larger granular lymphocytes) were refractory to the suppressive effects of PGE2 (3x10-8M to 3x10-6M). Our data demonstrate that: (1) stimulation of mononcelar cells with poly I:C is dependent on and regulated by relative levels of IFN produced by lymphocytes, and PGE2 produced by monocytes; and (2) that IFN-activated NK cells are protected from suppression by PGE2. (no Refs)

49. GRANULOPOIETIC INHIBITORS EXCRETED IN THE URINE OF A PATIENT WITH UTERINE CANCER AND MARKED LEUKOCYTOSIS.

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Exp Hematol; 10(4):315-325 1982

An inhibitor of colony stimulating factor (CSF) was found in normal human urine and in the urine of a patient with uterine cancer and leukocytosis. The amount of inhibitor excreted in the patient's urine was inversely related to the amount of CSF excreted. Since the patient's urine contained the inhibitor in large amounts, it was used for further characterization of the inhibitor. The inhibitor was separated from CSF by DEAE-cellulose column chromatography, and precipitated by ammonium sulfate at 30-80% saturation. The result of centrifugation of the inhibitor in a NaBr soln of d = 1.21 and extraction of its soln by chloroform suggested that it is not a lipoprotein. Furthermore, it was inactivated by heating at 60 C. Isoelectrofocusing resolved it into two peaks of pI 6.7-8.4 (pI-7 inhibitor) and pI 4.7-5.4 (pI-5 inhibitor), both of which showed an apparent mol wt of 80,000-90,000 upon Sephadex G-100 chromatography. Dose-response relation for CSF in the presence of the inhibitor(s) showed that the action of the inhibitor(s) was not due to specific inactivation of CSF. Both pI-5 and pI-7 inhibitor fractions showed mitogenic activity to mouse spleen cells in culture, and only slightly inhibited [3H]thymidine uptake in the PHA or LPS stimulated spleen lymphocytes. The result suggests that the granulopoietic inhibitor(s) obtained above is not a non-specifically cytotoxic substance(s). (Author abstract) (28 Refs)

50. A STUDY OF ADDED GM-CSF INDEPENDENT GRANULOCYTE AND MACROPHAGE PRECURSORS IN MOUSE SPLEEN INFECTED WITH MYELOPROLIFERATIVE SARCOMA VIRUS (MPSV).

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Exp Hematol; 10(4):373-382 1982

A subpopulation of granulocyte and macrophage precursors (GM-CFUc) differentiating in the agar colony technique of Bradley and Metcalf into mature granulocytes and macrophages, without the addition of granulocyte and macrophage colony stimulating factor (GM-CSF), can be detected in MPSV infected mice. These precursors were detected 5 days after virus infection, reaching a maximal concentration of 1/10,000 spleen or bone marrow cells, 25 days after viral infection. The number of the added GM-CSF independent GM-CFUc was linearly correlated with the number of seeded MPSV hematopoietic cells. No GM-CSF producing cells could be detected in the MPSV spleen using normal bone marrow GM-CFUc as responder cells. Study of the GM-CSF sensitivity of the GM precursors has demonstrated the existence of two GM-CFUc populations in the MPSV spleen: (a) a GM-CSF dependent population with a GM-CSF sensitivity similar to that of normal GM-CFUc, (b) a GM-CFUc population which differentiated in the absence of detectable amount of GM-CSF and of which differentiation was not affected by the addition of progressive amounts of GM-CSF. A possible model explaining these results is proposed. (Author abstract) (23 Refs)

51. GENERATION OF A COLONY STIMULATING FACTOR BY MURINE BONE MARROW CELLS THROUGH A SYNGERGISTIC EFFECT OF A TUMOR PROMOTING AGENT AND MITOGENS (MEETING ABSTRACT)

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Fed Proc; 41(3):563 1982

A tumor-promoting phorbol ester (TPA) 7,12-O-tetra decanoylphorbol-13-acetate induces very small colonies of macrophage/granulocyte progenitor cells (CFU-c) with no added colony stimulating factor (CSF) in soft agar bone marrow (BM) cultures. Although neither LPS (lipopolysaccharide) nor PHA (phytohemagglutinin) stimulate murine BM agar colonies, the addition of TPA to either one of these mitogens, but not to Con A (concanavalin A) or PWM (pokeweed mitogen), will induce typical CFU-c colonies. To elucidate this mechanism, experiments were designed to determine whether the effect was direct or modulated through the production of a CSF. Evidence of generation of a CSF was demonstrated by a) assaying supernatants from C57Bl/6 BM cells incubated for 24 hrs with TPA + LPS on BM cells from the LPS-resistant C3H/HeJ mouse strain; and b) generating supernatants from C57Bl/6 BM cells which were pulsed for 1-4 hrs with TPA + LPS, washed and resuspended and reincubated in fresh medium without TPA + LPS for an additional 24 hrs, and then assaying these supernatants on fresh C57Bl/6 BM cells. The resulting CFU-c colonies in agar cultures supplemented with each of the above supernatants suggests the existence of a CSF. This CSF is sensitive to trypsin digestion and is not inactivated by antiserum to L-cell CSF. Further definition of the mechanism of the TPA + mitogen stimulation, as well as the cell populations responsible, are currently being studied. (no Refs)

52. DIFFERENTIATION OF K-562 CELLS: INDUCTION BY THYMOSIN FRACTION 5 (MEETING ABSTRACT)

Ichiki AT, Lozzio BB, Lozzio CB, Bamberger EG
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Fed Proc; 41(3):795 1982

K-562 cells differentiate along the erythrocytic, granulocytic, and monocytic series; hence K-562 cells are considered to be multipotential leukemic cells. These cells express both specific leukemia associated antigens (LAA) and Fc receptors. We initiated a study employing monoclonal antibody preparations to determine the presence of other surface membrane antigens on the K-562 cells. When evaluated with the Cytofluorograf for the binding of F(ab')2 fragment of goat anti-mouse IgG, K-562 cells from 6 or 7 day-old cultures did not bind anti-HLA-A,B,C, anti-HLA-DR, OKT10 (reactive with thymocytes, BM terminal transferase positive cells, BM precursor B cells, and myeloblasts), OKT11 (reactive with large thymic blasts), or anti-monocyte antibody. Over 90% of the cells bound the five monoclonal anti-K-562 antibody preparations of differing specificities. The incubation of cells with thymosin fraction 5 for 24 hours led to changes in the expression of some surface antigens. The number of HLA-A,B,C expressing cells was increased but the number of OKT10 binding cells was only noticeably increased. There was also an increased intensity of the expression of LAA. The fact that the incubation of the K-562 cells with thymosin fraction 5 resulted in the expression of HLA-A,B,C suggests that these cells could be induced to differentiate along the lymphocytic series. (no Refs)

53. A BIOCHEMICAL VARIANT OF T-CELL GROWTH FACTOR (TCGF) PRODUCED BY A CUTANEOUS T-CELL LYMPHOMA CELL LINE (MEETING ABSTRACT)

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Fed Proc; 41(3):855 1982

Cell lines of mature T-cell origin established from patients with cutaneous T-cell lymphoma-leukemia have been shown to be constitutive producers of TCGF (L-TCGF). Active L-TCGF can be eluted from the plasma membranes of these cells. The biological activity of L-TCGF is similar to that of TCGF derived from PHA (phytohemagglutinin)-stimulated normal lymphocytes (N-TCGF): both support long-term growth of T-cells which have undergone prior lectin or antigen stimulation and have no effect on unstimulated T-cells. However, N- and L-TCGF possess disparate biochemical properties. Isoelectric focusing of N-TCGF produces multiple peaks of activity in the region of pH 6 to 8. Focusing of L-TCGF yields a single sharp peak with a pI of 4.5. During DEAE (diethyaminoethyl)-Sepharose anion exchange N-TCGF elutes in a molarity range of 0.07 to 0.1 M NaCl; L-TCGF dissociates at 0.2 M NaCl. Biogel P-60 gel filtration yields estimated molecular weights of 24,000 and 27,000 respectively. Treatment with neuraminidase, alkaline phosphatase, or a mixture of neuraminidase and endoglycosidases does not alter the isoelectric point, molecular weight or biological activity of L-TCGF, indicating that enzymatically vulnerable sialic acid, phosphate or glycosyl moieties are not involved in the variation. The nature and significance of this biochemical variant remain unknown. (1 Ref)

54. THE RELEASE OF GRANULOCYTE-MACROPHAGE COLONY STIMULATING FACTOR (CSF) BY LEUKAEMIC BLASTS: A MARKER OF MONOCYTIC DIFFERENTIATION (MEETING ABSTRACT)

Hinterberger W, Neumann E, Bettelheim P, Paukovits WR
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VI Meeting of the European Association for Cancer Research, October 12-15, 1981, Budapest, Hungary. The European Association for Cancer Research 275 pp., 1981.

In normal hemopoiesis, the release of CSF is a function of the monocyte-macrophage line and of activated T-cells. Blast cells from 30 patients with acute leukemia were analyzed by a set of morphological cytochemical, immunological and functional tests and the release of CSF was assayed by use of the agar cloning assay. The release of CSF proved to be restricted to blasts with monocytic or myelomonocytic differentiation. Malignant T-cells did not release CSF. Leukemic CSF was capable of inducing normal and leukemic stem cells to grow. Gel-chromatography (Sephadex G-75) from normal, monocyte-derived CSF and leukemic CSF from 4 patients disclosed a non-identity of elution profiles, in that each case differed from normal and also from each other. Thus, blasts with monocytic differentiation retain the capacity of their normal counterparts to release CSF. CSF may therefore serve to detect monocytic differentiation in acute leukemic blasts. (no Refs)

55. GROWTH FACTORS FROM PLATELETS, MONOCYTES AND ENDOTHELIUM - THEIR ROLE IN CELL PROLIFERATION (MEETING ABSTRACT)

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Conference on Cell Proliferation, Cancer and Cancer Therapy, February 17-19, 1982, New York, New York New York Academy of Sciences; :A3 1982

Growth factors have been isolated and purified that may play roles in embryogenesis; growth and development; cell turnover in adult tissues, cell proliferation in response to injury; and growth of some tumors. Growth factors derived from platelets, monocyte/macrophages and endothelial cells appear to play roles in response to injury. Platelet derived growth factor (PDGF) has been highly purified, is very potent (6 ng/ml equivalent to 5% whole blood serum) and binds to a specific cell surface high affinity receptor with an apparent Kd of 10-11M. Receptors for PDGF range from 600,000 per cell (a line of high receptor 3T3 cells) to 50,000 per cell (arterial smooth muscle) to no receptors (A431 cells and arterial endothelium). The PDGF receptor appears to be a peptide of approximately 164,000 molecular weight. Endothelial derived growth factor (EDGF) and monocyte/macrophage derived growth factor (MDGF) appear to be different from each other and from PDGF. Each of these growth factors has a potentially important role in the proliferation of mesenchymally derived cells in response to injury. (no Refs)

56. INVESTIGATION ON GRANULOCYTE-MACROPHAGE COLONY STIMULATING ACTIVITY (CSA) RELEASED BY HUMAN LEUKEMIC BLAST POPULATIONS (MEETING ABSTRACT)

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No affiliation given
10th International Symposium for Comparative Research on Leukemia and Related Diseases, August 31-September 4, 1981, Los Angeles, California. International Association for Comparative Research on Leukemia and Related Diseases 187 pp., 1981.

The growth of granulocyte-macrophage colony forming cells (CFUC) requires CSA, which, in normal hemopoiesis, is a product of monocytes, macrophages and T-cells. Blast cells from 30 patients with acute leukemia were analyzed by a set of immunological and functional tests and the release of CSA was measured by use of the agar cloning assay employing human marrow cells as target. The release of CSA proved to be restricted to blast populations with monocytic or myelomonocytic differentiation. In addition, the amount of CSA paralleled the intensity of cellular capacities regularly expressed during monocytic differentiation (esterase stain, lysocyme, adherence on solid surfaces). Malignant T-cells did not release CSA. Leukemic CSA was capable of inducing colony growth in normal and leukemic marrows: Normal marrow, activated with leukemic CSA, disclosed granulocytic, macrophage-and mixed colony formation. In cultures of leukemic marrows, a high degree of autostimulation was observed, when the blasts of the patients were capable of releasing CSA. Gel-chromatography (Sephadex G-75) from normal, monocyte derived CSA and leukemic CSA from 4 patients disclosed a non-identity of elution profiles, in that each case differed from normal and also from each other. In one patient, remission bone marrow was cultured on two occasions using normal leukocyte feeder layers and stored, autologous 'leukemic' CSA for activation: Cultures activated by feeder layers disclosed 25 and 30 CFUC/ 10(5) bone marrow cells, respectively. However, autologous, 'leukemic' CSA failed to activate the remission marrow on both occasions but induced a considerable number of colonies in parallel cultures with normal marrows. (no Refs)

57. MACROPHAGE AND T CELL-DERIVED FACTOR-MEDIATED REGULATION DURING TUMOR GROWTH: RESPONSIVE LYT SUBPOPULATIONS AND INTERLEUKIN LEVELS (MEETING ABSTRACT)

Elgert KD, Black SP, Burger CJ
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9th International RES Congress, February 7-12, 1982, Davos, Switzerland. American Reticuloendothelial Society 68 pp., 1982.

Normal and tumor-bearing host (TBH) BALB/c mice were used to assess if alteration in interleukins (IL) contributed to TBH immune hyporeactivity. This was tested by evaluating titratable macrophage (M) factor regulation of Lyt subpopulations, and examining further along the immune cascade sequence by determining levels of IL-2 and IL-3. Anti-Ly sera separated Lyt+ cells were examined for mixed lymphocyte reactivity in the presence of normal and TBH M supernatants. Blastogenesis of normal and TBH Lyt 1+ cells was inhibited at low dilutions of both normal and TBH M supernatants. At greater dilutions, TBH M supernatants only enhanced TBH Lyt 1+ cells not their normal host counterparts. Similar results were seen with the reverse experiment. Low and high dilutions of normal and TBH M supernatant inhibited and enhanced normal Lyt 2+ cells, respectively. A like pattern of suppression and enhancement was exhibited by TBH M supernatants for TBH Lyt2+ cells, however normal M supernatant only en-

hanced. No interferon or IL-2 activity was detected. There were low levels of IL-1 activity, however TBH M supernatant contained slightly higher activity than its normal host counterpart. Supernatants from 24 hr concanavalin A-induced normal and TBH spleen cells were tested for IL-2 and IL-3 activity. In a 28 day tumor growth kinetic study, IL-2 activity dropped 59% between Days 8 and 10 and was undetectable at Day 28. IL-3 activity also decreased by 59% between Days 4 and 8, and 12% of its activity was detectable on Day 38. Admixture of purified IL-2 and IL-3 induced normal spleen cells to a higher level of blastogenesis. IL-2 reconstituted hyporeactive TBH spleen cells to normal basal levels, however IL-3 further suppressed TBH spleen cell proliferation. Taken together, the paralysis of TBH T cell responses may be due to qualitative impairments of immune amplifying factors. (no Refs)

HORMONES/ STEROIDS/ PROSTAGLANDINS AS GROWTH EFFECTORS

58. MECHANISMS OF INHIBITION OF MOUSE MYELOID LEUKEMIC CELL DIFFERENTIATION BY PROSTAGLANDIN F2ALPHA.

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Gan; 73(2):175-183 1982

Mouse myeloid leukemia M1 cells were induced to differentiate in vitro into macrophages and granulocytes by various inducers, including dexamethasone. Prostaglandin F2alpha inhibited the inductions by dexamethasone of phagocytic and lysozyme activities in M1 cells. Prostaglandin F2alpha stimulated the production of differentiation-inhibiting activity (I-activity) in M1 cells. I-activity production by prostaglandin F2alpha was decreased by simultaneous treatment with actinomycin D (5 ng/ml) but not with 5-fluoro-2'-deoxyuridine (10 ng/ml). The I-activity was inactivated by heating (70 C, 20 min) or by treatment with trypsin but not with mixed glycosidases or ribonuclease, suggesting that I-activity was due to a proteinous substance(s). B-Type prostaglandins also stimulated I-activity production, whereas A-, E- and D-type ones did not. Induction of I-activity by prostaglandin F2alpha was suppressed by simultaneous treatment with prostaglandin E2. Retinoic acid stimulated the synthesis and release of prostaglandin F2alpha and production of I-activity in M1 cells. Indomethacin completely inhibited induction of I-activity by retinoic acid. On the basis of these result, the relationship between I-activity production and prostaglandin F2alpha production is discussed. (Author abstract) (39 Refs)

59. INTERACTION OF CHALONES AND GLUCOCORTICOID HORMONES IN REGULATION OF CELL DIVISION.

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Biull Eksp Biol Med; 93(2):51-52 1982

Interactions between the chalones and hydrocortisone (HC) were studied in C3HA mice with transplanted hepatoma 22a. Intact or adrenalectomized mice received a single or repeated injections of HC alone or in combination with chalone-containing liver extract. Analysis of cell proliferation in hepatoma 22a cells showed that chalone alone causes a 63.7% inhibition of mitotic activity, compared with 34.1% inhibition after single HC injection, 50.1% inhibition after repeated HC injection, and 61.0-72.4% inhibition after administration of chalone in combination with HC. Inhibitory effect of chalone was less pronounced in the adrenalectomized mice (45.2% inhibition of mitotic activity, compared with 63.7% inhibition in intact rats). These findings indicate that the expression of the inhibitory effect of chalone requires the presence of a certain level of adrenal cortex hormones in the blood. It was suggested that the type and the degree of interaction of chalones and glucocorticoid hormones depends upon the type of the target tissue. (12 Refs) (In RUS)

60. COMPARISON OF EFFECTS OF MEDROXYPROGESTERONE ACETATE (MPA) AND TAMOXIFEN (TAM) ON THE CELL GROWTH OF HUMAN BREAST CANCER CELL LINE (MCF-7) (MEETING ABSTRACT)

Nomura Y, Matsui K, Kanda K, Hamada Y, Tashiro H
Department of Breast Surgery, National Kyushu Cancer Center Hospital, Fukuoka, Japan
International Symposium on Medroxyprogesterone Acetate, February 24-26, 1982, Geneva, Switzerland. Farmatalia Carlo Erba 91 pp., 1982.

The effects of medroxyprogesterone acetate (MPA) on the cell proliferation of a hormone-responsive human breast cancer cell line (MCF 7) were studied with the colony formation method on the plastic dish, and compared with those of antiestrogen, tamoxifen (TAM) and steroid hormones. After the cells were cultured for 3 days in the medium with 10% FCS (fetal calf serum) from which estrogens had been removed by the dextran-coated charcoal method, 400 cells were plated on the 60 mm dish. 10-9M to 10-5M of MPA, TAM, estradiol-17 beta (E2), progesterone (P), testosterone (T), or dexamethasone (D) was added to the medium. The plating efficiency (PE) of the cells was suppressed dose-dependently by the addition of MPA. T, or D, irrespective of the presence or absence of E2. The inhibitory effect of TAM was hampered by the presence of E2. P showed no effects on the PE of MCF-7 cells. In two human breast cancer cell lines established in our laboratory, MPA and TAM showed different behaviors. The effects of MPA on the cell growth of MCF-7 cells may be different from those of TAM, or P. (no Refs)

61. HUMAN PROLACTIN AND HUMAN GROWTH HORMONE ALTER CELL SHAPE AND PROLIFERATIVE RATE OF HUMAN BREAST CANCER CELLS IN CULTURE (MEETING ABSTRACT)

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Proc Am Assoc Cancer Res; 23:933 1982

The role of prolactin and growth hormone in the etiology of human breast cancer is unclear. Therefore, the biological effects of these hormones on a human breast cancer cell line, T47D, were examined. T47D cells contain a high concentration of receptor sites that bind human prolactin(hPRL) and human growth hormone(hGH). When T47D cells were maintained on plastic substratum, they grew as a 'cobblestone' monolayer and assumed a flat, polygonal shape. However, when cultured in the presence of hPRL or hGH(10 ng/ml to 1 ug/ml), and 1 ug/ml each of hydrocortisone(H), insulin(I), and triiodothyronine(T3), T47D cells became round; the cells were loosely attached to the plastic substratum and they aggregated into large clusters. Such changes were not observed if only

hPRL or hGH were present, or if only HIT3 were present, indicating that hPRL and hGH require the synergism of the other three hormones(HIT3) to induce the shape change in T47D cells. Another lactogenic hormone, ovine prolactin, produced a similar effect as hPRL and hGH, whereas non-lactogenic hormones such as ovine growth hormone and human luteinizing hormone had no effect on the T47D cells. As well as having an altered shape, T47D cells proliferated twice as fast in serum-free medium supplemented with HIT3 only; T47D cells failed to proliferate in serum-free medium not supplemented with hormones. (1 Ref)

62. TUMOR CELL GROWTH FACTOR(S) ASSOCIATED WITH AN ESTROGEN-RESPONSIVE RAT MAMMARY TUMOR GROWING IN VIVO (MEETING ABSTRACT)

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Proc Am Assoc Cancer Res; 23:936 1982

Growth factors have been identified in extracts of MTW9/PL rat mammary tumor cells growing in vivo in response to estrogens. MTW9/PL tumors from intact females and from both estrogen treated castrated females and males had equivalent growth activity. Tumor extract protein added to serum-free DME at 10 ug/ml promoted significant MTW9/PL growth; 100 to 200 ug/ml replaced 75 to 100% of MTW9/PL serum requirement. Molecular properties of the tumor associated growth activity appear to be distinct from mammary growth factor activities in rat uterine, kidney, and pituitary tissues. The tumor activities show apparent molecular weights of 80,000 and 50,000 daltons on G-100 Sephadex and by heat, protease, and pH treatment, appear to be proteins. These activities are not due to residual serum and are not replaceable by known growth factors or hormones. Estrogens appear to influence directly growth factor activity level in MTW9/PL tumors since ovariectomy led to tumor regression and activity lower than that of tumors which are hormone autonomous or are growing in intact females. Our data suggest: 1) MTW9/PL tumor growth factors are related to estrogen levels in vivo; 2) autonomous tumors may produce growth factors continuously; 3) transformation from normal mammary gland to the neoplastic state includes the appearance of tumor associated growth factor. (no Refs)

63. SECRETION OF CALCITONIN AND PARATHYROID HORMONE BY EMBRYONAL CARCINOMA CELLS: VARIATIONS WITH DIFFERENTIATION (MEETING ABSTRACT)

Evain D, Binet E
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Pediatr Res; 15(12):1552 1981

Differentiation of teratocarcinoma cells in culture allows us to study certain aspects of the early embryogenesis. The adenylate cyclase system of cultured F9 cells is specifically stimulated by calcitonin. Differentiation into endodermal cells by treatment with retinoic acid is followed by a loss of calcitonin responsiveness and the appearance of parathyroid hormone stimulation of the cyclase system. In addition radioimmunological and biological active forms of calcitonin are secreted by F9 cells in the culture medium. Along with differentiation with retinoic acid a progressive decrease of calcitonin secretion is observed: after 4 days of treatment calcitonin is no longer detectable. But at this stage of differentiation a parathyroid hormone-like substance is radioimmunologically and biologically detectable. These specific changes in hormonal secretion and receptivity can be used as markers of differentiation. These results also suggest a possible role for Calcitonin and Parathyroid hormone-like activities during the initial steps of embryogenesis. (no Refs)

64. HORMONES AS ETIOLOGICAL AGENTS IN NEOPLASIA.

Furth J
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Cancer - A Comprehensive Treatise Vol. 1. Etiology - Chemical and Physical Carcinogenesis. Second Edition. Becker FF, ed. New York, Plenum Press, 714 pp., 1982.

Hormones as etiological agents in neoplasia are discussed. The basic properties of human hormones are reviewed, and general considerations in the detection of hormonal activity are listed. Brief general comments are presented on tumors in peripheral areas illustrating the diversity of pathogenic mechanisms by which hormones can produce tumors. Peripheral tumors are of two kinds: those affecting non-hormone-secreting organs, which do not feed back (as with the mammary gland), and those affecting peripheral hormone-secreting organs, which do. The best example of the former is the mammary tumor, and good examples of the second type are thyroid and ovarian tumors. Other tumors which are hormonally related include adrenal tumors which are unique in that they can give rise to four or more types of tumors - estrogen, androgen, glucocorticoid, and mineralocorticoid secreting - and in that they have different types of inductive mechanisms. Renal cortical carcinomas are induced by estrogens in male hamsters. Human placental lactogen stimulates carcinogenesis in young rats. It promotes mammary tumorigenesis during pregnancy, both directly and indirectly, through its promotion of progesterone secretion by the ovary. Thyrocalcitonin is the hypocalcemic polypeptide hormone secreted by the medullary cells of the thyroid. These cells can give rise to medullary thyroid carcinoma. (286 Refs)

65. CROSS-LINKED ENVELOPE FORMATION IN CULTURED HUMAN KERATINOCYTES: MODULATION BY HYDROCORTISONE AND VITAMIN A (MEETING ABSTRACT)

Cline PR, Rheinwald JG, Rice RH
Harvard School Public Health and Sidney Farber Cancer Institute, Boston, MA
Toxicologist; 2(1):91 1982

It has been found that 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) inhibits growth in culture of certain human keratinocyte lines derived from squamous cell carcinomas and that this inhibition is antagonized by hydrocortisone. In the present work, we have examined modulation by hydrocortisone and vitamin A of cross-linked envelope formation, a distinctive marker of keratinocyte differentiation. Cells of the carcinoma line SCC 13 were grown on a lethally irradiated 3T3 feeder layer in Dulbecco-Vogt Eagle's medium supplemented with 5% fetal calf serum and examined soon after reaching confluence for competence to form ionophore-inducible envelopes. Under these conditions, 10% or fewer of the cells were competent to form envelopes, whereas 40% to 60% of the cells grown with added hydrocortisone were competent. When the cells were grown and passaged in medium supplemented with charcoal extracted fetal calf serum, however, 60% of the cells were competent to form envelopes even without added hydrocortisone; when grown in the presence of treated serum and retinyl acetate, only 10% of the cells were competent, but with

addition of hydrocortisone as well, 60% were competent. These results are interpreted to mean that low concentrations of retinyl acetate in untreated fetal calf serum suppress cellular competence to form cross-linked envelopes, and that hydrocortisone is capable of antagonizing this effect. (no Refs)

66. TRI-IODOTHYRONINE (T(3)) STIMULATES THE GROWTH OF MORRIS HEPATOMA CELLS GROWN IN CULTURE.

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Biochem Biophys Res Commun; 105(4):1611-1617 1982

Cell cultures from transplanted Morris Hepatoma number 44 and host liver from female Buffalo rats were grown in medium containing 10% fetal calf serum (FCS) depleted of iodothyronines. The growth of liver cells was reduced by a max of 20% under such conditions, while that of the hepatoma cells was inhibited approx 40%. Addition of 200 nanoM tri-iodothyronine (T3) to the medium restored growth rate to control levels in both liver and tumor cells. Addition of 200 nanoM T3 to control medium (total concentration of T3 400 nanoM), did not significantly augment the rate of liver or tumor cell replication. Differences in cell growth rate between iodothyronine depleted and control media were significant different after 120 hr for liver and 24 hr for tumor cells. Thymidine kinase (TK) activity paralleled cell replication rate. A consistent and significant difference in TK activity compared to control values was observed only in cells grown in depleted medium after 48 hr of incubation. TK activity of hepatoma cells exceeded values of liver cells by a factor of 30-50 in all experiments. TK activity was markedly stimulated in cells grown in depleted medium supplemented with 200 nanoM T3 and control medium supplemented with 200 nanoM T3 compared to cells grown in control medium. Corresponding rates of hepatoma cell replication in such media did not significantly exceed those of cells grown in control media. Thus, T3 exerts a direct effect on the in vitro growth of hepatoma cells. Application of this method for studying the effect of hormones on receptor concentration and DNA and protein synthesis of neoplastic and normal tissue is suggested. (18 Refs)

67. A POSSIBLE ROLE OF HORMONES ON HEPATOCYTE AND HEPATOMA CELL PROLIFERATION (MEETING ABSTRACT)

Coetzee ML, Ove P
Department of Anatomy and Cell Biology, University of Pittsburgh, Pittsburgh, PA
Anat Rec; 202(3):33A 1982

Evidence is provided that proliferation of hepatocytes and transplantable Morris hepatoma 7777 might to some extent be regulated by triiodothyronine (T3), the adrenals and/or a specific serum protein, the levels of which are correlated with serum T3 levels. We have reported elevated levels of the serum protein following partial hepatectomy and a single injection of T3 and both are correlated with increased hepatic DNA synthesis. Both the cell proliferation of hepatoma 7777 and the circulating levels of the serum protein were decreased in thyroidectomized rats. When serum protein levels were elevated as a result of removal of 70% of the liver or a single injection T3, prior to transplant, accelerated hepatoma growth was observed. A tumor implanted into the back rather than in the thigh can be easily excised after 10 days with little injury to the animal. When this original transplant was surgically removed, serum protein levels rise rapidly and remain elevated for some time. A 2nd hepatoma transplant now grows at an accelerated rate. Both serum T3 and thyroxine (T4) levels are extremely low following thyroidectomy, but T3 levels are dramatically increased in hepatoma-bearing animals, while T4 levels remain constant. Adrenalectomy, performed 24 or 48 hr prior to partial hepatectomy, enhanced the response of increased DNA synthesis. On the other hand, dexamethasone, injected 48 hr prior to partial hepatectomy, completely inhibited the dramatic burst of hepatic DNA synthesis that usually occurs 20 hr post-operatively. (1 Ref)

CYCLIC NUCLEOTIDE/ POLYAMINES/ NUCLEOTIDES AS GROWTH EFFECTORS

68. PROTAMINE IS AN INHIBITOR OF ANGIOGENESIS.

Taylor S, Folkman J
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Nature; 297(5864):307-312 1982

Protamine is shown to be a specific inhibitor of angiogenesis. The compound inhibits the capillary proliferation observed in embryogenesis, inflammation, certain immune reactions and the growth of solid tumors. (Author abstract) (33 Refs)

69. DIBUTYRYL CAMP AND NERVE GROWTH FACTOR INDUCED DIFFERENTIATION OF HUMAN NEUROBLASTOMA (MEETING ABSTRACT)

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Med Pediatr Oncol; 9(5):526 1981

Human neuroblastoma tumors spontaneously differentiate into ganglioneuroma in vivo, causing them to regress spontaneously. We are investigating the mechanisms involved in differentiation by studying the effects of dibutyryl cAMP and nerve growth factor (NGF) of cAMP binding proteins (cAMP BPs) and protein kinases (PKs) in human neuroblastoma cell lines. One mM dibutyryl cAMP and NGF (250ng/ml) increased cAMP BPs by 2.5 and 1.5-fold. dibutyryl cAMP activiated cAMP dep-PKs in vivo, in contrast, NGF did not activate PKs. cAMP were identified by the photoactivation of cytoplasmic proteins plus 8-N(3)-32(P) cAMP and their analysis on 1-di and 2-di gels. 1-di-gels show 3 major cAMP BPs: 54,000 MW (R11) regulatory subunit of PK11, 51,000 MW ('R11) a new cAMP BP and 48,000 MW (R1) regulatory subunit of PKI. Dibutyryl cAMP increased R1 by 3-fold and NGF increased 'R11. 2-di-gels show 4 major cAMP BPs: R11 (54,000 MW, pI4.6), 'R11 (51,000 MW, pI5.0), R1 (48,000 MW, pI5.3) and R (48,000 MW, pI5.4) and 3 minor 'brain-specific' cAMP BPs number 2 to 4 (55,000 MW pI5.6-6.0). Dibutyryl cAMP increased R, induced a new protein 'R (48,000 MW, pI5.5) and BPs number 1 to 4. NGF increased BPs number 2-4. R1, R and 'R seem to be multiple forms of regulatory subunit of PKI and R11 and 'R11 of regulatory subunit of PKII. Whether all the multiple forms of cAMP BPs are associated with catalytic subunit of PKs is not known. Dibutyryl cAMP or NGF induces differentiation of neuroblastoma and induced cAMP BPs may play a role in cellular differentiation. (no Refs)

70. POLYAMINE METABOLISM IN MALIGNANT TUMORS.

Slatkin SP, Berezov TT
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Vestn Akad Med Nauk SSSR; (3):10-21 1982

The metabolism of polyamines (PA) was studied in random-bred albino rats with hepatoma 27 induced by diethylnitrosamine and in C3HA mice with hepatomas 22a, 61, 60, 48, and 46 induced by o-aminoazotoluene. PA content in hepatoma tissue was significantly greater than that in the normal liver. In all hepatomas except hepatoma 22a. The rate of ornithine carboxylation was significantly greater than that in the normal liver. All hepatomas were characterized by a virtual absence of diamine oxidase (DAO) activity. Significant increase of PA content and the increase of ornithine decarboxylase activity were observed 4-5 mo after the initiation of carcinogen administration, while the decrease of DAO activity was observed 1.5-3 mo after the initiation of the experiment. These findings indicate that the changes in the rate of PA oxidation can be considered as the primary manifestations of hepatocarcinogenesis, while the changes in ornithine decarboxylation reflect the tumor progression or dedifferentiation of tumor cells. (56 Refs) (In RUS)

COFACTORS/ NUTRITIONAL GROWTH FACTORS

71. THE EFFECT OF GLUTATHIONE ON AFLATOXIN B1 INDUCED TUMORS (MEETING ABSTRACT)

Novi AM, Florke R, Stukenkemper M
Department of Pathology, Dusseldorf University, Dusseldorf, W. Germany
Conference on Cell Proliferation, Cancer and Cancer Therapy, February 17-19, 1982, New York, New York New York Academy of Sciences; :A7 1982

Previous results from this laboratory demonstrated that reduced glutathione (GSH) administered to rats bearing aflatoxin B1 (AFB-1)-induced liver tumors caused regression of tumor growth. Evidence is reported here that the intact GSH molecule rather than its degradation product cysteine, is required by the neoplastic tissue to undergo regression of malignancy. Rats bearing AFB1-induced liver tumors were given a single oral dose of (35S) GSH and the uptake and subcellular distribution of radioactivity in various tissues was investigated. In the liver, most of 35S was recovered in the acid-soluble pool as GSH. Furthermore, a correlation between the amount of administered GSH and its recovery by the neoplastic tissue was observed, a result suggesting that the antitumor effect of GSH may be dose dependent (1 Ref)

72. EFFECTS OF RETINOIC ACID ON DIFFERENTIATION OF CHORIOCARCINOMA CELLS IN VITRO.

Chou JY
Pregnancy Res. Branch, Natl. Inst. Child Health and Human Development, NIH, Building 6, Room 128, Bethesda, MD, 20205
J Clin Endocrinol Metab; 54(6):1174-1180 1982

Choriocarcinoma cells maintain multiple hormonal functions in culture. We have found that these cells secreted no immunoreactive pregnancy-specific beta1-glycoprotein (PSbetaG), a placental protein. Choriocarcinoma cells can be induced to synthesize low levels of PSbetaG by retinoic acid, 8-bromo-cAMP (8BrcAMP), cholera toxin, methyl-isobutylxanthine (MIX), and 5-bromo-2'-deoxyuridine (BrdUrd). The simultaneous addition of retinoic acid along with 8BrcAMP, cholera toxin, or MIX gave synergistic induction of PSbetaG. The simultaneous addition of retinoic acid and BrdUrd failed to give even additive induction. In addition to stimulating PSbetaG production, retinoic acid increased the production of hCG and its alpha-subunit (hCGalpha) by choriocarcinoma cells. The simultaneous addition of retinoic acid along with 8BrcAMP, cholera toxin, or MIX gave additive induction for hCG and hCGalpha. Passage of choriocarcinoma cells in medium containing retinoic acid induced a stable altered phenotype characterized by elevated levels of PSbetaG, hCG, and hCGalpha. These retinoid-treated choriocarcinoma cells remained responsive to 8BrcAMP or compounds that increase intracellular cAMP concentrations and to BrdUrd; the production of PSbetaG and hCG was greatly stimulated by 8BrcAMP, cholera toxin, or MIX, and the production of hCG and hCGalpha was greatly inhibited by BrdUrd. However, the production of hCGalpha was only slightly induced by these cAMP modulators, and the production of PSbetaG was not increased by BrdUrd. (Author abstract) (29 Refs)

73. COMPARATIVE FUNCTIONAL CHANGES IN HL-60 CELLS INDUCED TO DIFFERENTIATE BY RETINOIC ACID AND DIMETHYLSULFOXIDE (MEETING ABSTRACT)

Hemmi H, Breitman TR, Keene BR, Frank DA
National Institutes of Health, Bethesda, MD, 20205
Fed Proc; 41(3):683 1982

The human promyelocytic cell line, HL-60, differentiates to morphologically and functionally mature granulocytes by incubation with a wide variety of compounds. In this study, we have compared functional changes (measured by nitroblue tetrazolium (NBT) reduction, hexose monophosphate shunt (HMPS) activity and superoxide anion production) accompanying the progression of HL-60 promyelocytes to myelocytes, metamyelocytes, and banded and segmented neutrophils when induced by two structurally diverse compounds: all-trans-retinoic acid (RA) and dimethylsulfoxide (DMSO). With both inducers there was a direct correlation between the percentage of cells reducing NBT and the percentage of cells that had morphologically matured to at least the myelocyte stage. However, both HMPS activity and superoxide anion production of RA-induced HL-60 were at least two-fold lower than DMSO-induced cells at comparable levels of morphological maturation. These functional activities of DMSO-induced cells were similar to those of normal peripheral blood granulocytes. Thus, while RA is one of the most potent physiological inducers of HL-60 morphological maturation and is effective at the physiological concentration of 30 nM, it produces cells that are defective in at least two functional parameters. A possible explanation is that RA induces a 30-fold increase in plasma membrane nicotinamide adenine dinucleotide glycohydrolase activity. (no Refs)

74. EFFECT OF L-METHIONINE DEPRIVATION ON THE PROLIFERATIVE ACTIVITY OF NORMAL AND LEUKAEMIC MARROW CELLS IN VITRO (MEETING ABSTRACT)

Eridani S, Sawyer B, Villa S, Tisdale M
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Br J Cancer; 44(2):278 1981

A comparison has been made between normal and leukemic marrow with regard to the capacity to incorporate methyl-3HdT (deoxythymidine) into acid-insoluble material, after incubation in media lacking L-methionine and supplemented with L-homocysteine. A lower incorporation was shown by leukemic cells, than normals ones, with a significant difference. Marrow cells of leukemic patients in remission behave as normal cells. This difference is not due to inability of leukemic cells to synthesize L-methionine from homocysteine, because an increased incorporation can be seen in the presence of homocysteine when methionine concentration in culture becomes limiting. Further experiments suggest that such high methionine requirement might be due to a decreased transport capacity by leukemic cells: all marrows show the same linear uptake for the whole range of concentrations tested, but the maximum uptake velocity is lower in leukemic cells. Restriction by different means of the supply of methionine to leukemic cells might play a role in the control of myeloproliferative disorders. (no Refs)

75. INHIBITION OF TUMOR GROWTH WITH LOW DIETARY PROTEIN AND WITH DIETARY GARLIC EXTRACTS (MEETING ABSTRACT)

Criss WE, Fakunle J, Knight E, Adkins J, Morris HP, Dhillon G
Department of Human Nutrition and Food, Howard University, Washington, DC, 20059
Fed Proc; 41(3):281 1982

Dietary preparations from fresh garlic bulbs (Allium sativum) inhibited the growth of Morris hepatoma 3924A. Diets containing 5%, 15%, or 25% casein (each +/-5% lypholized garlic extracts) were fed to rats without and with inoculated tumor tissues. The results were as follows: Low dietary protein decreased tumor growth from 60-70%. All diets containing 5% garlic yielded decreased tumor growth from 10-25%. The dietary garlic extract was non-toxic as no changes were observed in levels of serum albumin, albumin/globulin ratio, urinary creatinine, liver or spleen size, or animal growth. Similar types of studies were performed by subcutaneously injecting a garlic extract into rats bearing this tumor. In the latter studies, tumor growth was slowed by 30-50%, but the injection treatment resulted in toxicity. Since these garlic extracts contain a potent inhibitor of guanylate cyclase, the only enzyme known to produce the growth stimulating substance, cGMP, it is possible that the inhibition of tumor growth by garlic feeding may result from the reduction of tumor cGMP levels. (no Refs)

EXOGENOUS AGENTS AFFECTING DIFFERENTIATION

76. INHIBITION OF PHORBOL ESTER-RECEPTOR BINDING BY A FACTOR FROM HUMAN SERUM.

Horowitz AD, Greenebaum E, Nicolaides M, Woodward K, Weinstein IB
Div. Environmental Sciences and Cancer Center, Inst. Cancer Res., Columbia Univ., New York, NY, 10032
Mol Cell Biol; 2(5):545-553 1982

The mechanisms by which a factor isolated from human serum (SF) inhibits phorbol-12,13-dibutyrate (PDBu) binding in intact monolayer cultures of the rat embryo cell line CREF N and in a subcellular system containing membranes of these cells, were studied. In addition the release induction of arachidonic acid and choline from prelabeled cellular phospholipids by PDBu was compared to that of the SF. The SF may perform a protective role in host susceptibility to carcinogenesis. (26 Refs)

77. PHORBOL ESTER INDUCTION OF LEUKEMIC CELL DIFFERENTIATION IS A MEMBRANE-MEDIATED PROCESS.

Cooper RA, Braunwald AD, Kuo AL
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Proc Natl Acad Sci USA; 79(9):2865-2869 1982

Phorbol esters are potent inducers of macrophage-like differentiation in the HL-60 promyelocytic leukemia cell line. The sequence of events by which they bring about this transition is poorly understood. However, it is known that phorbol esters bind to the surface membrane of HL-60 cells and to various other cells as well. Our studies were directed toward determining the biologic importance of this membrane association. [3H]Phorbol dibutyrate (PBu2) was specifically bound by HL-60 cells with a Kd of 23 nM and with 1.9 x 10(5) binding sites for [3H]PBu2 per cell. There was no internalization of bound [3H]PBu2. Specific binding was fully reversible upon washing in fresh medium, and [3H]PBu2 added thereafter bound normally to its receptor. Within 10 min of binding, PBu2 stimulated [14C]choline incorporation into phosphatidylcholine, with a rapid return to normal upon removal of the PBu2. Membrane-bound PBu2 progressively inhibited DNA synthesis, with 70% inhibition by 8 hr. This process was interrupted if the PBu2 was removed and little recovery of DNA synthesis occurred in previously inhibited cells. Between 8 and 16 hr, PBu2 induced adherence of cells to plastic, but only in those cells in which phosphatidylcholine synthesis was stimulated, and this process was also interrupted if PBu2 was removed prior to 16 hr. Similarly, nonspecific esterase, which develops after 72 hr of incubation, was induced in cells exposed to PBu2 for the initial 16 hr but not in cells exposed for 5 hr. These studies demonstrate that phorbol esters exert their effects while retained at the cell surface. Inhibition of cell growth and the acquisition of surface and enzymatic properties that characterize macrophages are separable events, even of which proceeds through a receptor-mediated, transmembrane process. The stimulation of phosphatidylcholine synthesis appears to be a part of that process. (Author abstract) (34 Refs)

78. MECHANISMS FOR THE EFFECT OF PHORBOL MYRISTATE ACETATE ON PROADIPOCYTE DIFFERENTIATION (MEETING ABSTRACT)

Yun K, Scott RE
Mayo Clinic, Rochester, MN, 55905
Fed Proc; 41(3):330 1982

The role of phorbol myristate acetate (PMA) as a tumor promoter is thought to be mediated in part by its ability to block cell differentiation. However in some cells, PMA promotes differentiation instead of blocking differentiation. The mechanisms which mediate these antagonistic effects of PMA and their significance in tumor promotion have not been explained. In an attempt to answer these questions, we studied the control of 3T3 T proadipocyte differentiation and the effects of PMA. First, it is shown that proadipocyte differentiation is regulated by an equilibrium of five separate processes; three processes promote differentiation and two processes block differentiation by promoting proliferation. Next, it is shown that PMA can effect both the positive and negative aspects of differentiation control. Under certain experimental conditions the net effect of PMA is to prevent differentiation whereas under different conditions PMA either has no net effect on differentiation or slightly facilitates differentiation. These actions of PMA are shown to be modulated by microenvironmental conditions and by the metabolic state of the proadipocytes. We suggest that similar factors may determine which cells serve as the target for the tumor promoting effect of PMA in vivo. (no Refs)

79. LIPID ACCUMULATION BY AORTIC CELLS FOLLOWING EXPOSURE TO TUMOR PROMOTING PHORBOL ESTERS IN VITRO (MEETING ABSTRACT)

Miller GJ, Ferrara JA
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Fed Proc; 41(3):269 1982

The tumor promoting phorbol esters have been shown to modulate the phenotype of a wide variety of cells grown in vitro. We have previously shown that exposure of primary culture, newborn, murine (ICR) aortic cells to the potent tumor promoter phorbol-12-myristate-13 acetate (PMA) causes these cells to accumulate oil-red-0 positive material in their cytoplasm thereby producing a morphology similar to atherosclerotic 'foam-cells'. The present study demonstrates that 1) this morphologic transition is dependent on the density of the cells during the time of exposure to the tumor promoter; 2) the transition occurs in proportion to the tumor promoting ability of the specific phorbol analog being used and 3) a simple metabolite of PMA (myristic acid) is not responsible for the transition. In addition the various promoting phorbol analogs tested affect the plateau density but not the growth rate or plating efficiency of the aortic cells. Staining with the lipase-lead sulfide technique reveals that the lipid accumulated by these cells is composed largely of triglyceride. This has been confirmed by 14C acetate precursor incorporation and thin layer chromatography. Further study of this system may provide insight into the pathogenesis of early lipid accumulation in atherosclerosis. (no Refs)

80. INDUCTION OF DIFFERENTIATION IN HUMAN MONOBLAST LINE U937 BY PHORBOL ESTER (PMA), LYMPHOKINE (LK) AND OTHER PROTEINS: SURFACE ANTIGENS AND NONSPECIFIC AND ANTIBODY-DEPENDENT (ADCC) TUMORICIDAL ACTIVITY (MEETING ABSTRACT)

Punjabi CJ, Ralph P, Litcofsky PB
Sloan-Kettering Institute, Rye, NY, 10580
Fed Proc; 41(3):563 1982

Culture of U937 for 3-6 days with certain agents induced macrophage antigens Mac-1 and 3 recognized by monoclonal antibodies, Fc and C receptors, latex bead phagocytosis, and ADCC to tumor targets. Active agents included PMA, LK (from PHA (phytohemagglutinin) or PWM (pokeweed mitogen)-stimulated spleen or blood leukocytes) and 2 nonlymphoid conditioned media (CM) containing myeloid colony stimulating activity (GCT from GIBCO and bladder carcinoma line 5637), but not mock LK, IL-2 containing CM from JURKAT line, or other CM. PMA also induced nonspecific killing of mouse 18-8 pre-B leukemia targets assayed by 20 hr (125)IUdR (iododeoxyuridine) release. Induction by all agents of the macrophage properties was associated with growth cessation of U937, suggestive of a terminal differentiation process. The activity in 5637 CM was destroyed by boiling, and had a mwt of 20-40,000. These experiments show that a) the same monocyte type can mediate both nonspecific and antibody-dependent lysis of tumor targets, b) exogenous agents and endogenous factors other than LK can induce ADCC, c) the U937 line can be used as a model for differentiation or maturation of human monocytic cells. (no Refs)

81. PHORBOL-ESTER INDUCED CHANGES IN CHRONIC LYMPHOCYTIC LEUKEMIA CELL PHENOTYPE (MEETING ABSTRACT)

Shawler DL, Glassy MC, Wormsley SB, Royston I
University of California at San Diego, School of Medicine, La Jolla, CA, 92093
Fed Proc; 41(3):794 1982

Primary cultures of chronic lymphocytic leukemia (CLL) cells can be induced to differentiate by a phorbol ester (TPA, 12-O-tetradecanoylphorbol-13-acetate). Using flow cytometry, the effect of TPA was monitored by studying alterations in the following lymphocyte markers: T65 (a T cell antigen also found on CLL cells); HLA-Dr; surface Ig; cytoplasmic Ig; Lyt3 (an antigen associated with the sheep erythrocyte rosette receptor); and mouse erythrocyte rosette formation (MR). Three CLL phenotypes were studied; common CLL (sIg+, HLA-Dr+, MR+ T65, Lyt3-); T cell CLL (sIg-, HLA-Dr-, MR-, T65+, Lyt3+); and a rare case of CLL (sIg-, HLA-Dr+, MR+, T65+, Lyt3-). TPA increased the expression of T65 by a similar magnitude in all 3 CLL phenotypes. In both the common and the rare phenotype of CLL, TPA increased the expression of HLA-Dr, decreased MR, and induced cIg, but it did not induce Lyt3. In the rare phenotype, TPA also induced sIg. This suggests that this rare type of CLL is derived from the same B cell lineage as common CLL. In addition to the increased expression of T65, TPA also increased the expression of Lyt3 in T cell CLL, but did not induce any B cell markers. Our data suggest that common CLL is a proliferation of any early, immature B cell. The presence of T65 on the surface of common CLL cells suggest that T65 may be a progenitor cell antigen found on immature B cells in addition to thymocytes and peripheral T cells. (no Refs)

82. EFFECTS OF 12-O-TETRADECANOYLPHORBOL-13-ACETATE (TPA) ON RAT PHEOCHROMOCYTOMA (PC12) CELLS: INTERACTIONS WITH EPIDERMAL GROWTH FACTOR AND NERVE GROWTH FACTOR.

End D, Tolson N, Yu MY, Guroff G
Section Intermediary Metabolism, Lab. Developmental, Neurobiology NIH, Bethesda, MD, 20205
J Cell Physiol; 111(2):140-148 1982

The interactions of 12-O-tetradecanoylphorbol-13-acetate (TPA) with nerve growth factor (NGF) and epidermal growth factor (EGF) were investigated to determine if TPA produced a generalized uncoupling of the cellular responses of these two peptide growth regulators. TPA was a potent inhibitor of 125I-EGF binding to PC12 cells, but did not inhibit the binding of 125I-NGF to these cells. TPA shared several common actions with EGF and NGF. The incorporation of 3H-choline into acid-precipitable material was stimulated by TPA, EGF and NGF within 2 hr after addition of the compounds. The effects of TPA were additive to the effects of NGF and EGF, and phorbol did not alter 3H-choline incorporation. The growth factors, and TPA also induced ornithine decarboxylase activity within 5 hr after their addition. The effects of TPA were additive to those of EGF and NGF. TPA, EGF and NGF selectively stimulated the phosphorylation of a chromatin-associated non-histone protein, and the effects of TAA were additive to those of the growth factors. When PC12 cells were treated with TPA in combination with either NGF or EGF, the cells rapidly extended very limited processes. Although these extensions could achieve substantial lengths, they nevertheless seemed morphologically distinct from NGF-induced neurites. TPA appeared to produce its similar effects via a receptor system distinct from the EGF receptor system. (45 Refs)

83. MACROPHAGE DIFFERENTIATION OF HUMAN PRECURSOR B-CELL LINE BY PHORBOL ESTER AND COLONY-STIMULATING FACTOR.

Hiraoka A, Kubota K, Preisler HD, Minowada J
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Blood; 59(5):997-1000 1982

A human round cell line, KLM-2, is considered to be precursor B-cell by immunologic surface marker analysis and histochemical studies. The effect of 12-O-tetradecanoyl phorbol-13-acetate (TPA) and/or colony-stimulating factor (CSF) on KLM-2 cells was investigated. KLM-2 cells became adherent to the bottom of the flask on day 2 after liquid culture with TPA, and the number of macrophage-like adherent cells (mAC) with pseudopodia showed a TPA dose-dependent increase in the peak level on day 3 or 4. The mAC had a phagocytic activity. The suspending cells were positive for alpha-naphthyl acetate esterase (NAE) up to about 50% in culture with TPA. A TPA concentration of 100 ng/ml was optimal to provide a maximum number of mAC and NAE-positive suspending cells. Particularly, such effects of TPA on KLM-2 cells were markedly accelerated in the presence of CSF. These findings indicate macrophage differentiation of KLM-2 cells in the presence of TPA and CSF. KLM-2 cells may be a bipotential stem cell line differentiating into B-cell and monocyte-macrophage lineages. (Author abstract) (15 Refs)

84. CORRELATION BETWEEN SENSITIVITY TO TUMOR PROMOTION AND SUSTAINED EPIDERMAL HYPERPLASIA OF MICE AND RATS TREATED WITH 12-O-TETRA-DECANOYLPHORBOL-13-ACETATE.

Slaskin EE, Gray T, Barrett JC
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Carcinogenesis; 3(4):403-407 1982

The role of sustained hyperplasia in tumor promotion has been reexamined in light of our previous report that the Syrian hamster, which is insensitive to two-stage epidermal carcinogenesis initiated by 7,12-dimethylbenz[a]anthracene (DMBA) and promoted by 12-O-tetradecanoylphorbol-13-acetate (TPA), does not respond with epidermal hyperplasia after multiple treatments with the promoter. Epidermal hyperplasia of several other species and strains of animals, which vary in their responsiveness to TPA-mediated tumor promotion, was examined after single or multiple treatments with the tumor promoter TPA. The strains examined were CD-1, C57B1, Balb/c, DBA, and F 344. A striking difference was observed in the response of the different animal species to single versus multiple exposures to the promoter. When the effects of single versus multiple doses of TPA were compared, every possible combination of responses was observed, including potentiation, adaptation, and no change. Thus, the results of a single exposure could not be used to predict the response with multiple treatments. While no correlation was observed between sensitivity to tumor promotion and hyperplasia induced by a single exposure of TPA, a good correspondence was found between sensitivity to tumor promotion and TPA induced sustained hyperplasia after 4 wk of treatment. The role of sustained hyperplasia in tumor promotion and the necessity to study persistent effects induced by TPA are discussed. (23 Refs)

85. THE EFFECTS OF RETINOIC ACID AND A TUMOR PROMOTER, 12-O-TETRADECANOYLPHORBOL-13-ACETATE, ON LYMPHOCYTE PROLIFERATION.

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Dept. Microbiology, Cell Biology and Biophysics, Pennsylvania State Univ., University Park, PA, 16802
Carcinogenesis; 3(4):409-413 1982

The tumor promoter, 12-O-tetradecanoylphorbol-13-acetate (TPA) inhibits DNA synthesis in allogenic, mixed cultures of bovine lymphocytes. Retinoic acid, an antagonist of TPA in in vivo skin promotion, was tested for its ability to counteract the effect of TPA on lymphocyte proliferation. Retinoic acid or related compounds, retinol or retinol acetate, did not reverse or prevent the inhibitory effect of TPA. Instead retinoic acid also inhibited DNA synthesis in mixed lymphocyte cultures. On the av, about 8 uM retinoic acid inhibited the mixed lymphocyte response by 50%. The mitogenic response of lymphocytes to phytohemagglutinin (PHA) was also inhibited by retinoic acid. The degree of inhibition depended both on the concentration of PHA and of retinoic acid. Therefore, in regard to bovine lymphocytes, retinoic acid depresses DNA synthesis in both allogenic and lectin stimulated responses. Such suppression should be taken into account in the use of retinoic acid in chemotherapy. (Author abstract) (29 Refs)

86. TUMOUR-PROMOTING AND NON-PROMOTING PRO-INFLAMMATORY PHORBOL ESTERS AS HUMAN LYMPHOCYTE MITOGENS (MEETING ABSTRACT)

Edwards M, Taylor SE, Evans FJ, Nouri AM, Gordon D
Department of Pharmacognosy, The School of Pharmacy, London, England
J Pharm Pharmacol; (33,Suppl):55P 1981

TPA (Tetradecanoylphorbolacetate) a potent tumor-promoting and pro-inflammatory agent is the only T-lymphocyte mitogen known which can dispense with the macrophage requirement for proliferative responses. We examined the effects of nine related compounds as lymphocyte mitogens by monitoring 3H-thymidine incorporation by Ficoll-Paque separated human blood mononuclear cells. The co-mitogenic effects of the compounds on Mixed Lymphocyte Responses (MLR) using lymphocytes from 2 unrelated donors were studied in the same way. The pro-inflammatory but non-tumor promoting diesters 12DPPAA and 12DPAA were only weakly active as mitogens. The corresponding C-13 monoesters had similar activities to TPA, suggesting that the polarity of C-20 is important for hydrogen-bonding to cell membrane proteins. As the parent alcohol phorbol was inactive, this suggested that esterification at C-13 is necessary for activity, possibly by increasing lipid solubility. Sapintoxin A had a similar activity to TPA but its 5-OH analogue, Sapintoxin B, had reduced activity. The possibility of intermolecular hydrogen bonding between the C-5 and C-20 OH groups could reduce the availability of the C-20 position for binding. The 4 alpha-analogue of Sapintoxin A was inactive as a lymphocyte mitogen demonstrating the importance of the ring AB-trans configuration for activity. All of the 12-deoxyphorbol esters, including the non-promoting diesters acted as co-mitogens in the MRL system, being more potent than TPA. These results suggest that either a different mechanism is involved in co-mitogenicity or, more likely, that mitogenicity involves a two-stage signal, only one of which is required in co-mitogenicity. The non-promoting esters may be capable of fulfilling one signal but only partially triggering the second signal. (3 Refs)

87. PROLIFERATION OF HUMAN LYMPHOCYTES INDUCED BY A SERIES OF TUMOUR-PROMOTING PHORBOL ESTERS (MEETING ABSTRACT)

Dewdney RS, Ladd C, Soper CJ
University of Bath, Bath, England
J Pharm Pharmacol; (33,Suppl):56P 1981

As part of an investigation into the mechanisms of action of tumor promoters we have examined the abilities of a series of phorbol esters to induce human lymphocyte proliferation, in order to determine if the activities of these compounds as lymphocyte mitogens correlate with their potencies as tumor promoters. Mononuclear cells, predominantly lymphocytes, were separated from heparinized human peripheral blood by sedimentation on Ficoll-Isopaque gradients. The cells were washed in phosphate buffered saline and then washed and resuspended at 10(6) cells/ml in a modified RPMI 1640 medium supplemented with 10% fetal bovine serum. The cell suspension was treated with phorbol ester and dispensed in 0.1 ml volumes into the walls of microtitre plates. The plates were incubated for 72 h at 37C in a humidified 5% CO2 atmosphere. Proliferative stimulation of lymphocytes was assessed by cellular 3H-thymidine uptake. 3H-thymidine 1u Ci/ml was added to each microculture for the final 24h of incubation. The microcultures were harvested onto glass fiber filter discs and the entrained cells washed successively with distilled water, 5% trichloroacetic acid and methanol. The discs were dried thoroughly in glass scintillation vials. Five ml of scintillation cocktail (PPO 6g, POPOP 0.05g/l toluene) was added to each vial. Counts per minute (cpm) of incorporated 3H-thymidine from a minimum of five replicate microcultures were obtained for each phorbol ester dose. For the purposes of comparison, the lowest concentration of a particular ester inducing significant 3H-thymidine uptake is used as a measure of the mitogenic activity of that ester. In dose-response studies phorbol esters with pronounced mitogenic activity gave similar maximum levels of stimulated 3H-thymidine uptake. Our data suggest that the mitogenic activities of these esters correlate with their potencies as tumor promoters. (no Refs)

88. SELECTIVE TOXICITY OF DIMETHYLSULFOXIDE FOR HEMATOPOIETIC CELLS OF HUMAN EMBRYONAL LIVER.

Makartseva TM
Res. Inst. Hematology and Blood Transfusion, Kiev, USSR
Tsitol Genet; 16(1):10-14 1982

To confirm the findings that dimethylsulfoxide (DMSO) affects differentiation of normal myeloid and of leukemic cells, the effect of DMSO on the hematopoiesis was studied in the organ culture of human embryonal liver. Incubation with 1% DMSO resulted in marked inhibition of the myeloid hematopoiesis and active proliferation of the stromal cells. The increased proliferation rate was associated with the changes of the cell adhesiveness. (11 Refs) (In RUS)

89. IMMUNOSUPPRESSIVE PROPERTIES OF POLAR ORGANIC COMPOUNDS THAT INDUCE CELLULAR DIFFERENTIATION IN FRIEND ERYTHROLEUKEMIA CELLS.

Suthanthiran M, Rubin AL, Novogrodsky A, Stenzel KH
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Transplantation; 33(5):534-540 1982

We studied the effect of several polar organic compounds, known to induce erythroid differentiation in Friend leukemia (FL) cells, on in vitro human lymphocyte responses and on skin graft survival in mice. The short chain fatty acids, butyric acid (BA), propionic acid (PA), valeric acid, and the polar organic solvents, dimethyl sulfoxide, dimethylformamide, and dimethylacetamide, all mediated significant inhibition of alloantigen-induced proliferation and generation of cytotoxic T lymphocytes (CTLs) in human primary and secondary mixed lymphocyte culture (MLC) reactions. Hexamethylenebisacetemide, another potent inducer of differentiation in FL cells, also mediated significant suppression. Inhibition of MLC with polar organic compounds was accomplished at concentrations known to induce differentiation in FL cells and that are not cytotoxic to peripheral blood mononuclear cells. In distinct contrast, agents that are structurally related to BA, but that do not induce differentiation in FL cells, such as caproic acid, beta-OHBA, gamma-amino BA, and isobutyric acid, did not exhibit immunosuppressive properties. Pokeweed mitogen-driven polyclonal B cell activation was also suppressed by agents that induce erythroid differentiation in FL cells. In addition to potent in vitro immunosuppresive properties, supplementation of drinking water with BA or PA resulted in prolongation of full-thickness skin grafts in DBA/2 (H-2d) to C57BL/6 (H-2b) donor-recipient combinations. Our findings indicate that polar organic compounds that induce differentiation in FL cells are potent immunosuppressive agents. (Author abstract) (21 Refs)

90. CRANIAL NEURAL CREST CELLS: INHIBITION OF DIFFERENTIATION IN CULTURE.

Greenberg JH, Glimelius B, Knudson
Lab. Developmental Biology and Anomalies, Natl. Inst. Dental Res., NIH, Bethesda, MD, 20205
Adv Neurol; 29:105-113 1981

The effects of dimethylsulfoxide, 5-bromo-2'-deoxyuridine, retinoic acid, infection with Rous sarcoma virus, and temperature on differentiation of cultured chick neural crest cells are described. Exogenous agents that interfere with neural crest cell differentiation may act at a variety of sites, and may sometimes mimic genetic or environmental influences that occur in vivo and lead to birth defects. (25 Refs)

91. DNA LIGASE ACTIVITY IN EXTRACTS OF MOUSE ERYTHROLEUKEMIA (MEL) CELLS DURING DIFFERENTIATION (MEETING ABSTRACT)

Scher BM, Scher W, Waxman S
Mt. Sinai School of Medicine, New York, NY, 10029
Conference on Cell Proliferation, Cancer and Cancer Therapy, February 17-19, 1982, New York, New York New York Academy of Sciences; :AP-19 1982

DNA ligase and DNAase activities were determined in cell-free extracts prepared from MEL cells grown for 5 days in the absence or in the presence of 1.8% (v/v) dimethylsulfoxide (DMSO). DMSO treatment induces erythrodifferentiation in these cells and is accompanied by hemoglobin production, by a limitation in cell multiplication, and by nuclear condensation. DNA ligase activity in extracts from DMSO-treated cells was 167, 95, 22, 12, and less than 4% of the activity in extracts from untreated cells after 19, 43, 67, 95, and 113 hr of DMSO treatment, respectively. The major DNAase activity was inhibited by G-actin and was, therefore, presumed to be DNAase I. DNAase I-like activity increased slightly with DMSO treatment. These changes were not seen in a variant cell line resistant to DMSO induction. The DNA ligase changes may be related to differentiation; possibly to the limitation in cell multiplication and/or nuclear condensation. (no Refs)

92. CORRELATION BETWEEN MEMBRANE PERMEABILITY AND INDUCTION OF ERYTHROID DIFFERENTIATION BY DMSO IN FRIEND VIRUS TRANSFORMED ERYTHROLEUKEMIA CELLS (MEETING ABSTRACT)

Dube SK, Yamase T
No affiliation given
10th International Symposium for Comparative Research on Leukemia and Related Diseases, August 31-September 4, 1981, Los Angeles, California. International Association for Comparative Research on Leukemia and Related Diseases 187 pp., 1981.

We have measured the uptake of phosphate, leucine, uridine, thymidine and deoxyglucose by FEL (Friend erythroleukemia) cells before and after induction with DMSO (dimethyl sulfoxide). The results showed a dramatic decrease in the uptake of these molecules by induced cells amounting to a drop of about 70% of the control. Our experiments on 32P phosphate incorporation in these cells showed that newly synthesized globin mRNA appears in the cytoplasm about 10 hours after addition of DMSO. To determine whether the decrease in membrane permeability temporally coincided with globin mRNA induction, we measured the uptake of phosphate, leucine, etc at hourly intervals after addition of DMSO. A very interesting duality of effect was observed. At first hour time point, there was an increase in the uptake of these molecules and this increase continued up to about 8 hours. After that time the uptake showed first signs of decrease and this was almost concomitant to the initiation of induced globin mRNA synthesis. To further strengthen the interpretation of the uptake data, we used amphotericin B, which is known to increase membrane permeability. We found that if amphotericin B and DMSO are added together, the inducing effect of DMSO is totally wiped out, ie, no globin synthesis is observed. These results, therefore, strongly argue for a correlation between decrease in membrane permeability and induction of erythroid differentiation in FVC (Friend virus complex) transformed proerythroblasts. (no Refs)

93. ANTI-TUMOR EFFECT AND TERMINAL DIFFERENTIATION INDUCING ACTIVITY OF PHOSPHODIESTERASE INHIBITORS (MEETING ABSTRACT)

Ambrus CM, Huberman E, Horoszewicz J, Leong SS, Biddle W, Montagna R, Gastpar H, Ambrus JL
Roswell Park Memorial Institute, Buffalo, NY, 14263
Proc Am Assoc Cancer Res; 23:894 1982

In previous studies we found that neoplastic human cells produce more cAMP phosphodiesterases than surrounding normal cells. It was thought that inhibitors of these enzymes may have selective anti-tumor effects. In normal and neoplastic human cell lines in culture inhibitors of cAMP phosphodiesterases were tested for anti-tumor effect by cell count and 3H thymidine incorporation. Cell lines studied include ES-1 malignant melanoma, DAUDI and HR-IK Burkitt's lymphoma, 6410 acute myeloid leukemia, MOLT-4F and CCRF-CEM acute lymphoid leukemia, HL-60 promelocytic leukemia, HT-29 colon adenocarcinoma. RT-4 transitional cell bladder carcinoma, LNCap prostatic carcinoma, BG-27 diploid foreskin fibroblasts and NC-37 normal B lymphocytes. The methylxanthine derivative pentoxifylline and the pyrimido-pyrimidine derivative RA-233 exhibited significant anti-tumor effect. This appears to be related to increase in cAMP levels. There was more effect in the lag phase of cell growth than in the logarithmic growth phase. There was more effect on neoplastic cells than on normal cells, particularly in the lower dose range. In certain cell lines RA-233 was significant terminal differentiation inducing effect by morphologic and enzymologic criteria. (1 Ref)

94. UPTAKE INTRACELLULAR DISTRIBUTION AND METABOLISM OF N-N1-DIACETYL-14C-PUTRESCINE (DDAB), A POTENT INDUCER OF FRIEND ERYTHROLEUKEMIA DIFFERENTIATION (MEETING ABSTRACT)

Canellakis ZN, Bondy PK, Gazitt Y
Veterans Administration Medical Center, West Haven, CT
Fed Proc; 41(3):682 1982

Previous publications have implicated ornithine decarboxylase and the polyamines in the induction of differentiation in Friend erythroleukemia (FL) cells. We now report the uptake and metabolism of DDAB in FL cells induced to differentiate by 10mM DDAB (1uCi/umole). The results indicate a gradual increase during induction in the amount of DDAB taken up by 5x10(7) cells within 24 hours to the cytosol (14.4 n moles), nuclear fraction (5.8 n moles) and membrane fraction (15.8 n moles). Concurrently, there is an increase in the extent of conversion of DDAB into the major metabolites, putrescine, spermidine and spermine. At 24 hours 40% of cytosolic DDAB is metabolized and appears as putrescine (11%), spermidine (15.4%) and spermine (2%). We also detected small amounts of

N-Monoacetyl putrescine (2%) and N-acetyl spermidine (1%). (no Refs)

95. REVERSAL BY ISAXONINE OF THE INHIBITORY EFFECT OF VINCRISTINE ON THE GROWTH OF NEURITES IN CULTURES.

Tarrade T
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Nouv Presse Med; 11(16):1233-1237 1982

It has been previously demonstrated that isaxonine increases neurite elongation of dorsal root ganglia in vitro. In purpose to demonstrate that this effect results of an action at the level of the cellular cytoskeleton, we have studied effects of isaxonine on neurite elongation inhibited by vincristine sulfate which is known to bind specifically on tubulin and consequently perturb microtubule assembly. Neurite elongation of dorsal root ganglia cultivated with vincristine (0.5 or 10-10M) is compared to neurite elongation of ganglia cultivated with vincristine plus isaxonine (10 7-10 1M). Results show that isaxonine prevents, at least partially, the effect of vincristine. The protective effect observed in vivo in the rat and in the human seems to indicate that isaxonine acts at the level of microtubules of nerve fiber. (Author abstract) (12 Refs) (In FRE)

96. INHIBITORY EFFECTS OF VARIOUS OXYGENATED STEROLS ON THE DIFFERENTIATION AND FUNCTION OF TUMOR-SPECIFIC CYTOTOXIC T LYMPHOCYTES.

Spangrude GJ, Sherris D, Daynes RA
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Transplantation; 33(5):482-491 1982

These studies were undertaken to determine the effect of various oxygenated sterols (representing a number of known cholesterol-derived photoproducts) on the generation (afferent) and function (efferent) of cytotoxic T lymphocytes (CTLs). Cell-mediated immune responses which result in the generation of both alloantigen-specific and syngeneic tumor-specific CTLs were evaluated. The results of our studies established the following points: (1) the afferent phase of an immune response is particularly sensitive to the effects of some cholesterol photoproducts while other oxidized sterol derivatives, known to exist in skin following UV light (UVL) exposure, have minimal influence on the generation of CTL responses; (2) the only known carcinogenic photoproduct of cholesterol (cholesterol alpha-oxide) is a very weak inhibitor of lymphocyte function; and (3) presentation of oxygenated sterols via liposome membranes is 10-fold more effective than their administration in soluble form; (4) 25-hydroxycholesterol and 20alpha-hydroxycholesterol were found to be the most potent inhibitors, causing a parallel depression in lymphocyte proliferation, CTL activity, and endogenous sterol biosynthesis, and (5) 7beta- and 7alpha-hydroxycholesterol functioned in a unique manner, causing an abrogation of CTL activity without depressing proliferative responses and endogenous sterol biosynthesis to a significant degree. These studies represent the first time that an attempt has been made to evaluate a possible biochemical basis for immune suppression known to precede experimental UVL carcinogenesis. Our results suggest that some, but not all, of the oxidation products of cholesterol found in skin following UVL irradiation exert an influence on immune responses. Derivatives of cholesterol could be partially responsible for the alterations in immunological potential of the UVL-exposed host. The induced presence of an inhibitor of CTL differentiation or function at a time following UVL tumor emergence may play a role in this process by providing an early tumor escape mechanism. (37 Refs)

OTHER RELATED STUDIES

97. INTERACTION OF CELLULAR AND HUMORAL FACTORS IN TUMOR SUPPRESSION (MEETING ABSTRACT)

Andreotti PE
Department of Microbiology and Immunology, Duke University Medical Center, Durham, NC
Control of Human Cancer III, March 6-7, 1981, Fort Lauderdale, Florida. North Ridge General Hospital 19 pp., 1982.

Tumor immunity is dependent upon the antigenic expression of the tumor cell, and is potentially modulated by the secretion of factors by tumor cells which affect immunocompetence. Macrophages and natural killer (NK) lymphocytes are primarily important in cell mediated tumor immunity. These cell types can express spontaneous cytolytic activity against tumor cells, and can be further activated by the immunologic secretion of humoral factors including macrophage activation factor (MAF), interferon (IFN), and prostaglandins which comprise one class of arachidonic acid derivatives now recognized to be important immunoregulatory factors. The production of other lymphokines and monokines by T lymphocytes and macrophages, respectively, are also involved in tumor immunity. Cytotoxic T lymphocytes (CTL) may play a role in tumor suppression, particularly viral associated neoplasms of which there are few, if any known for man. The importance of antibodies in tumor suppression, and enhancement, is well recognized in spite of the difficulty in defining tumor specific antigens with serological methods. Antibody mediated effector mechanisms in tumor suppression may involve humoral components of the complement cascade, macrophages, and killer (K) lymphocytes armed with cytophilic antibody. Other less well defined humoral effectors, such as tumor necrosis factor (TNF), may be involved in tumor suppression. (no Refs)

98. CURRENT STATUS OF BIOLOGICAL RESPONSE MODIFIERS (MEETING ABSTRACT)

Oldham RK
No affiliation given
Biologic Control of Human Cancer II, April 4-5, 1981, Fort Lauderdale, Florida. North Ridge General Hospital 10 pp., 1981.

The Biological Response Modifiers Program (BRMP) is a comprehensive program of the Division of Cancer Treatment, National Cancer Institute, involved in clinical and laboratory research with both extramural and intramural components to investigate, develop and bring to clinical trials potential therapeutic agents which may alter biological responses important in the biology of cancer growth and metastasis. The program was conceived as a focused approach in DCT to support further basic research in biological response modifiers and to rapidly apply potential leads from that research to the treatment of cancer in man. The classes of agents to be investigated in this program include immunoaugmenting, immunomodulating and immunorestorative agents, interferons and interferon inducers, lymphokines, cytokines and growth factors, thymic factors, tumor antigens and modifiers of tumor antigen cell surface components, antitumor antibodies, antitumor cells, tumor preventing agents, and maturation and differentiation factors. It is recognized that considerable research is underway in each of these areas but a focused, coordinated approach by the NCI may result in the rapid acquisition of knowledge and a more rapid application of information to the treatment of cancer. BRM predefined as those agents or approaches which modify the relationship between tumor and host by modifying the

host's biological response to tumor cells with resultant therapeutic effects. (no Refs)

99. ISOLATION AND CHARACTERIZATION OF MONOCLONAL ANTIBODIES DIRECTED AGAINST DIFFERENTIATION SPECIFIC CELL SURFACE ANTIGENS OF NORMAL AND LEUKEMIC MYELOID CHICKEN CELLS (MEETING ABSTRACT)

Kornfeld S, Graf T, Beug H
Deutsches Krebsforschungszentrum, Institut fur Virusforschung, Heidelberg, W. Germany
9th International RES Congress, February 7-12, 1982, Davos, Switzerland. American Reticuloendothelial Society 68 pp., 1982.

By immunization of mice with normal macrophages and myeloid leukemia cells transformed by the three acute avian leukemia viruses E26, AMV and MC29, which resemble myeloid cells at different stages of maturation, a series of monoclonal antibodies directed against differentiation-specific cell surface determinants could be isolated. These antibodies were characterized both by indirect immunofluorescence on erythroid, myeloid and lymphoid leukemia cell lines and on normal bone marrow cells, using double fluorescence in combination with rabbit antibodies specific for erythrocytes, immature erythroid cells, myeloblasts and macrophages, respectively. The monoclonal antibodies obtained were of the following types 1) Antibodies reacting predominantly with immature myeloid cells. 2) Antibodies which bind predominantly to mature macrophages. 3) Antibodies specifically reacting with mature and immature myeloid cells. 4) Antibodies directed against determinants shared by myeloid and B and/or T lymphoid cells. 5) Antibodies directed against determinants shared by myeloid and immature erythroid cells. 6) Antibodies reacting with all kinds of hematopoietic cells except erythrocytes. By quantitative binding assays using anti-macrophage or -myeloblast monoclonal antibodies, different numbers of surface determinants could be detected on myeloid cells of different degree of maturation. Some of these antibodies immunoprecipitated surface glycoproteins of 130 and 170 kd, and a nonglycosylated protein of 30 kd. These monoclonal antibodies may be valuable tools to further characterize both the leukemic cells transformed by different strains of acute avian leukemia viruses as well as their normal bone marrow target cells. (no Refs)

100. DIFFERENTIATION OF TERATOCARCINOMA CELLS (MEETING ABSTRACT)

Knowles BB
The Wistar Institute, Philadelphia, PA
Hybridoma; 1(2):175 1982

Monoclonal antibodies to murine stage-specific embryonic antigens (SSEA-1 and-3) have been used to define the pluripotent embryonal carcinoma (EC) stem cells of both murine and human teratocarcinomas and to monitor their differentiation in vitro and in vivo. SSEA-1, expressed beginning at the 8-cell stage of murine preimplantation embryos, becomes progressively restricted upon differentiation of the inner cell mass. SSEA-1 is expressed on murine EC cells, but not on those of the parietal endoderm, the most routinely observed differentiated derivative of EC cells in culture. SSEA-3, expressed on ova and cleavage stage embryos, is not expressed on the primitive ectoderm of the developing inner cell mass. Murine EC cells are SSEA-3 negative, however, human EC cells can be defined by their reaction with anti-SSEA-3. Differentiation of human EC cells in vitro results in a population which becomes SSEA-1 positive. Since SSEA-1 and SSEA-3 are carbohydrate antigenic determinants, their changing expression points to the regulation of glycosyltransferases and/or glycosidases during development and differentiation. (no Refs)

101. DIFFERENCES IN GROWTH REGULATION OF NORMAL AND TUMOR CELLS (MEETING ABSTRACT)

Pardee AB, Campisi J, Croy RG
Sidney Farber Cancer Institute, Boston, MA
Conference on Cell Proliferation, Cancer and Cancer Therapy, February 17-19, 1982, New York, New York New York Academy of Sciences; :A13 1982

Normal cells cannot initiate DNA synthesis yet complete their division cycle under inadequate external conditions. The sensitive biochemical event is proposed to be accumulation of protein which in adequate amounts permits entry into S phase, after about 2 hr, and completion of the cycle. Instability of the protein (half life about 2.5 hr) creates a dynamic state so that accumulation depends on rates of both synthesis and degradation. Neoplastic cells may show poorly regulated growth by synthesizing more of this protein or degrading it less rapidly, under conditions that limit normal cells' growth. Overproduction can result from more copies of structural gene per cell, adjacent promoter of high activity, or autoproduction of growth factors. Less rapid degradation could result from less protease activity, or from stabilizing modifications of the protein. Alterations of protein metabolism by these mechanisms have been described. Thus, diverse transforming agents acting via different mechanisms could affect a common regulatory process leading to neoplasia. (no Refs)

102. KINETICS OF PROLIFERATION OF SMALL INTESTINE EPITHELIUM AFTER CARMINOMYCIN ADMINISTRATION.

Fomina TI
Central Res. Lab., Medical Inst., Tomsk, USSR
Antibiotiki; 27(3):183-186 1982

To evaluate the mechanism of gastrointestinal toxicity of carminomycin (CM), mice (strain unspecified) were given a single ip injection of CM at LD50. Within different time periods after administration, the mice were sacrificed and proliferation of the epithelial cells in the small intestine was determined. CM produced a decrease of the mitotic activity and inhibition of DNA synthesis in the small intestine crypts. It was suggested that crypt exhaustion results in enhanced cell differentiation and thus in the impairment of the intestinal absorption. (10 Refs) (In RUS)

103. ARE THERE TWO DIFFERENT FORMS OF NEUROBLASTOMA?

Pahlman S, Grotte G
Wallenberg Lab., Box 562, S-75122 Uppsala, Sweden
Z Kinderchir; 35(2):62-63 1982

Neuroblastomas regress spontaneously in approx. 7% of patients, apparently by differentiation of tumor cells or by disappearance of tumor cells by cytolysis. Initial growth of these tumors may be due to an excess of growth factors or a deficiency in factors inducing differentiation. Tumors that regress spontaneously therefore may not involve genetic defects. Differentiation of neuroblastoma cells has been induced in vitro by 12-O-tetradecanoyl-phorbol-13-acetate. The existence of a more common malignant form and a less common benign form of neuroblastoma is suggested. (10 Refs)

104. DEGREE OF DIFFERENTIATION AND BLOOD VESSEL PROXIMITY IN B16 MELANOMA.

Paulus G, Hong LX, Atassi G, Buyssens N
Dept. Pathology, Univ. Antwerp, Universiteitsplein 1, B-2610 Wilrijk, Belgium
Virchows Arch [Cell Pathol]; 39(2):229-238 1982

Corded structures consisting of rows of viable tumor cells around a central blood vessel are present in a number of transplantable mouse tumors, including transplantable B16 melanomas. These tumors were used to assess, by stereological means at the EM level, the range of differentiation of the melanoma cells, according to their position in relation to the central blood vessel. The mitotic index was also determined for perivascular and peripheral tumor cells separately. Furthermore, the transition of peripherally located cells into necrotic tumor cells is described at the EM level. Results show an important increase in differentiation in peripheral tumor cells, whereas the mitotic index is highest in perivascular cells. Necrotic peripheral cells show features of apoptotic necrosis, together with necrosis of the ischemic type. Results indicate that both proliferation and differentiation of melanoma cells are related to their position around a central blood vessel, and that peripheral necrosis is not exclusively due to lack of oxygen. (Author abstract) (10 Refs)

105. IN VITRO TRANSFORMATION BY AVIAN ERYTHROBLASTOSIS VIRUS RECRUITS CELLS OF ERYTHROID LINEAGE WITH VARYING DEGREE OF DIFFERENTIATION.

Therwath A, Scherrer K
Dept. Microbiology, Biomedical Center, Box 581, S-751 23 Uppsala, Sweden
Int J Cancer; 29(5):555-558 1982

We have previously shown that the AEV infection of leukosis-free chickens provokes an erythroleukemia in the infected birds, and that the target cell for virus transformation is most likely an early pro-erythroblast comparable to the BFU-E. The virus infection correlates with a block in differentiation which was studied at a specific cellular gene level, namely that of the globin genes. In live birds, the viral transformation has been found to affect the transcriptional activity of two out of three adult globin genes and the phenotypic expression of the third. Indeed, the alphaa gene that is transcribed is silent as well, since its transcription product is fully eliminated in the nucleus following an 'abortive processing'. Chick bone marrow cells can also be transformed in vitro by AEV in which case the virus recruits, as targets, cells of the erythroid lineage belonging to a wider spectrum and hence in varying degrees of differentiation. In contrast to the results obtained with in vivo transformed cells, molecular hybridization with globin cDNA probes showed that in the erythroblasts transformed in vitro under tissue culture conditions all the three adult globin genes are transcriptionally active. Judging from our present results, we may propose that AEV transformation in vitro does not shut off but only modulates the already functioning erythroid cell differentiation program. (14 Refs)

106. IN VITRO STUDIES OF HUMAN PLURIPOTENTIAL HEMATOPOIETIC PROGENITORS IN POLYCYTHEMIA VERA. DIRECT EVIDENCE OF STEM CELL INVOLVEMENT.

Ash RC, Detrick RA, Zanjani ED
Dept. Medicine, Veterans Admin. Medical Center, Univ. Minnesota Sch. Medicine, Minneapolis, MN, 55417
J Clin Invest; 69(5):1112-1118 1982

Previous in vitro studies on committed hematopoietic progenitors have suggested that polycythemia vera (PV) is a clonal disorder arising in a pluripotential hematopoietic stem cell. In this study, recently developed technics for clonal assay of a human multipotential progenitor cell (CFU-GEMM) were used to assess the functional characteristics of CFU-GEMM in 19 PV patients. These studies showed: (a) increased numbers of detectable CFU-GEMM in blood and bone marrow samples of PV patients as compared with normal (p less than 0.002 and p less than 0.02, respectively); (b) erythropoietic differentiation of PV CFU-GEMM without exogenous erythropoietin (Ep) in culture (in marked contrast to CFU-GEMM of both normals and subjects with secondary erythrocytosis which require exogenous Ep for terminal hemoglobinization of their erythroid component), a property shown by experiments with an anti-Ep antiserum to be related to increased sensitivity of PV CFU-GEMM to Ep; (c) increased megakaryocyte formation by PV CFU-GEMM as compared with normal (p less than 0.025); and (d) a linear relationship, extrapolating to the origin, between CFU-GEMM detected and cell cultured. These studies demonstrate that at least two clinical features of PV, increased erythropoiesis and megakaryocytopoiesis, are reflected in corresponding functional characteristics of PV CFU-GEMM, and provide direct evidence of distinctive pluripotential stem cell activity in this disorder. (Author abstract) (24 Refs)

107. AN ORNITHINE DECARBOXYLASE-DEFICIENT MUTANT OF CHINESE HAMSTER OVARY CELLS.

Steglich C, Scheffler IE
Dept. Biology, B-022, Univ. California, San Diego, La Jolla, CA, 92093
J Biol Chem; 257(8):4603-4609 1982

We have selected a mutant of Chinese hamster ovary cells that is severely deficient in ornithine decarboxylase activity and is auxotrophic for putrescine. The mutant we obtained (C54) has only 3% of the max inducible ornithine decarboxylase activity of the parental Chinese hamster ovary cells and the rate of incorporation of [3H]ornithine into acid-soluble material is correspondingly reduced. The defect in the mutant was recessive in somatic cell hybrids. The mutant requires at least 10(-5) M putrescine in the medium to maintain a normal growth rate. Spermidine and spermine can also serve as a polyamine source, and very high (millimolar) concentrations of ornithine can support a normal growth rate. In the absence of polyamine supplementation the cells stop growing after about three population doublings and begin to die after 5 or 6 days. The intracellular concentrations of putrescine and spermidine are depleted after 24 and 48 hr, respectively. Spermine levels remain essentially constant for 4 days. Unexpectedly, the Km for ornithine of the enzyme from mutant cells was consistently somewhat lower than the Km for the enzyme from wild type cells. It is possible but not yet certain that the mutant is the result of a mutation in the structural gene coding for ornithine decarboxylase. (Author abstract) (52 Refs)

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CELL AND SERUM DERIVED GROWTH EFFECTORS

1. FACTORS THAT AFFECT HUMAN HEMOPOIESIS ARE PRODUCED BY T-CELL GROWTH FACTOR DEPENDENT AND INDEPENDENT CULTURED T-CELL LEUKEMIA-LYMPHOMA CELLS.

Tarella C, Ruscetti FW, Poiesz BJ, Woods A, Gallo RC (c/o Gallo) Lab. Cell Biology, NCI, NIH, Bethesda, MD, 20205
Blood; 59(6):1330-1336 1982

Some laboratory results and clinical situations suggest that human T cells may be important in the regulation of growth of hematopoietic cells. Since the discovery of T-cell growth factor (TCGF), systems are now available for the long-term specific in vitro propagation of mature normal or neoplastic human T cells, providing an opportunity to study the influence of T cells on hematopoiesis. Recently, 24 cell lines from patients with cutaneous T-cell lymphoma (CTCL) and T-cell acute lymphoblastic leukemia (T-ALL) were grown with TCGF and then assessed for release of humoral factors that affect hematopoiesis. Conditioned media (CM) from these cell lines were tested for erythroid burst-promoting activity (BPA)

and granulocyte colony-stimulating activity (CSA). BPA was detected in CM from 3/6 cultures of T-ALL patients and 4/6 CTCL cultures. CSA was found in the CM from 6/8 cultures of T-ALL patients, 7/12 CTCL cultures, and 3/4 CTCL cell lines that become independent of exogenous TCGF for growth. The CSA from several of the neoplastic T-cell cultures stimulated high levels of eosinophil colonies, a possible source of the eosinophilia seen in these patients. The ability of continuously proliferating human T lymphocytes, which retain functional specificity and responsiveness to normal humoral regulation, to produce factors that directly or indirectly stimulate myeloid and erythroid colony formation lends further credence to the role of T lymphocytes in regulating hematopoiesis. (Author abstract) (34 Refs)

2. LYMPHOKINES: A FORUM FOR IMMUNOREGULATORY CELL PRODUCTS.

Pick E, Landy M
Dept. Human Microbiology, Sackler Sch. Medicine, Tel-Aviv Univ, Ramat-Aviv, Tel-Aviv, Israel
Lymphokines 2: 313 pp., 1981.

Lymphokine (LK)s activities are discussed. A systematic screening for LK activities detectable in the supernatants of continuous lymphoid and myeloid cell lines is presented. Present knowledge of T-cell growth factor (TCCF) and prospects for future LK studies stemming from the discovery and rapidly expanding application of TCCF are considered. Other topics discussed include the following: the central role played by T- and B-cell growth factors in the primary antibody response in vitro, the immune response in vitro, the role of antigen-specific T-cell factors in both help and suppression, the role of soluble factors in macrophage-T-cell interaction, the mechanism of action of LK, the mechanism of action of WBC migration inhibitory factor, the biological universality of LK, cytokine-like mediators secreted by malignant cells, tumor necrosis factor, the design of a method for enumerating LK-secreting cells, and the significance of the presence of LK in the tissues of animals and humans. (852 Refs)

3. LYMPHOKINES: A FORUM FOR IMMUNOREGULATORY CELL PRODUCTS. VOLUME 3. LYMPHOKINES IN MACROPHAGE ACTIVATION.

Pick E, Landy M
Dept. Human Microbiology, Sackler Sch. Medicine, Tel-Aviv Univ., Ramat-Aviv, Tel-Aviv, Israel
Lymphokines 3: 450 pp., 1981.

The role of lymphokines (LK) in macrophage activation (MA) is discussed under the following headings: an introduction to MA, biochemical properties of activated macrophages, enhancement of phagocytosis-associated oxidative metabolism as a manifestation of MA, macrophage plasma membrane enzymes as differentiation markers of MA, a developmental concept for the heterogeneity of macrophages in response to LK and other signals, role of colony-stimulating factors as primary regulators of macrophage functions, lipopolysaccharide-unresponsive mice as a model for analyzing LK-induced macrophage differentiation in vitro, MA and resistance to intracellular infection, the role of cyclic nucleotides in the suppression of intracellular replication of Toxoplasma in LK-activated macrophages, cytotoxicity of activated macrophages for the multicellular parasite Schistosoma mansoni, the role of LK in granulomatous inflammations, the cytostatic and cytocidal effects of macrophages and their specificity for tumor cells, the killing of syngeneic tumor cells by guinea pig macrophages activated in vitro with lymphocyte mediators, tumor cytotoxicity by LK-activated macrophages: development of macrophage tumoricidal activity requires a sequence of reactions, induction of tumoricidal capacities in mouse and rat macrophages by LK, MA to cytotoxicity by the macrophage cytotoxicity (activating) factor, the effect of activated macrophages on tumor growth in vitro and in vivo, and requirement of transmethylation reactions for immune effector function. (1532 Refs)

4. MECHANISMS OF TCGF PRODUCTION BY CLONED T-CELL LINES: CHARACTERIZATION OF PRODUCER LYMPHOMA LINES AND KINETICS OF PRODUCTION.

Smith RT, Shimizu S, Norcross MA, Maino VC
Tumor Biology Unit, Dept. Pathology, Univ. Florida, Coll. Medicine, Gainesville, FL, 32610
Clin Immunol Immunopathol; 23(2):286-296 1982

The activation requirements and kinetics of production of T-cell growth factor are described for a stable, cloned T-lymphoma line, EL4-G-12. The line yields a product which has the biochemical and biological attributes of TCGF of normal cell origin examined thus far. Production is dependent upon a transmembrane signal which can be provided by known T-cell-activating lectins, and by antibody to a specific ligand on the Thy-1 molecule, if accessory cells or factors are provided. Production is rapid, beginning within 4 hr, and ceasing between 12 and 18 hr depending upon cell density, signal intensity, and culture conditions. DNA synthesis ceases as production begins, and release is not a result of cell death. It has not yet been determined whether rapid synthesis de novo, or activation and release of an inactive hormone precursor explains the production kinetics observed. (Author abstract) (17 Refs)

5. RELATIVE AND ABSOLUTE DEFICIENCY OF BONE MARROW ENDOGENOUS COLONY STIMULATING ACTIVITY IN ACUTE MYELOID LEUKEMIA: RELATION TO RESPONSE TO CHEMOTHERAPY.

Francis GE, Berney JJ, Wing MA, Tuma GA, Hoffbrand AV
Dept. Haematology, Royal Free Hosp., Pond St., London NW3 2QG, England
Leuk Res; 6(2):165-173 1982

Possible mechanisms behind the association between the different acute myeloid leukemia (AML) clonogenic cell clone size and colony stimulating activity (CSA)-sensitivity phenotypes and the response of the patient to induction therapy were investigated in marrow samples taken from 26 AML patients (21 at presentation and 5 post therapy). Fifty-two percent (11/21) of patients at presentation showed deficiency of endogenous CSA when assayed as relative endogenous CSA. In contrast, only 31% (5/16) showed absolute deficiency of endogenous CSA at presentation. Patients were classified into three groups: those with undetectable or very low endogenous CSA (less than 20% of the mean normal value), those with moderately reduced endogenous CSA below the lower limit of normal range, and those with normal or high endogenous CSA. The observed generalized trend indicated that the worse the deficiency of endogenous CSA, the worse the response to therapy. A statistically significant inverse correlation between the relative endogenous CSA and the number of courses of therapy required to achieve remission, and an inverse correlation, although not statistically significant, between the relative endogenous CSA and the change in marrow blasts after the first course of therapy were observed. Two out of the three patients in the severely CSA deficient group failed to achieve remission and the decline of blasts was very poor or absent in all three patients. Although the results suggested that an associ-

ation between relative endogenous CSA and response therapy exists, the results do not show the close correlation expected. (14 Refs)

6. BIOLOGY OF ESTABLISHED HUMAN LYMPHOMA CELL LINES (MEETING ABSTRACT).

Kaplan HS
Department of Radiology, Stanford University School of Medicine, Stanford, CA, 94305
In Vitro; 18(3,part2):319 1982

Beginning with the African Burkitt lymphomas, several types of diffuse lymphomas have now been established as permanent cell lines, whereas the nodular (follicular) lymphomas have proven refractory to in vitro cultivation. Limited success has been reported with the giant cells from patients with Hodgkin's disease. Clonal growth in semisolid media has also been observed, but may require special conditions. The cutaneous T cell lymphomas require supplementation with human T cell growth factor (TCGF), except for rare lymphomas which are constitutive TCGF procedures. The established cell lines have been extensively characterized with respect to cell surface markers and functional attributes. In contrast to banal EBV-transformed B-lymphoblastoid lines, they are all monoclonal, remarkably stable in phenotype, and most lack EBV genomes. The criteria of neoplasia in lymphoma cell lines differ in several important respects from those of neoplasms derived from anchorage-dependent cells. (no Refs)

7. A B LYMPHOMA CELL LINE PRODUCES GROWTH FACTORS FOR HEMOPOIETIC, LYMPHOID, AND MAST CELLS.

Clark-Lewis I, Schrader JW, Wu YY, Harris AW
Walter and Eliza Hall Inst. Medical Res., Royal Melbourne Hosp., Post Office, Victoria 3050, Australia
Cell Immunol; 69(1):196-200 1982

A cloned B lymphoma cell line (WEHI-231.1) released P-cell stimulating factor, a factor allowing the in vitro growth of presumptive mast cell precursors together with small amounts of T-cell growth factor and granulocyte-macrophage colony-stimulating factor. These factors were produced without deliberate stimulation of the B lymphoma cells. Lipopolysaccharide-stimulated nude mouse spleen cells also released low levels of P-cell stimulating factor and granulocyte-macrophage colony-stimulating factor. These results raise the possibility that B cells may produce regulatory factors. (Author abstract) (20 Refs)

8. FIBROBLAST INTERFERON INHIBITS THE GROWTH OF CFU-C (MEETING ABSTRACT).

Meyer P
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Onkologie; 4(5):285 1981

The question of whether an in vitro effect of fibroblast interferon on granulocytically committed precursor cells (CFU-C) can be detected was investigated. The test system used consisted of an agar bilayer with a feeder layer of granulocytes for production of colony stimulating factor (CSF) and an overlayer with the target cell population (buffy coat cells from the bone marrow of healthy human donors). Fibroblast interferon was utilized in amounts of 100, 1000, and 2000 units (U) per culture. The addition of interferon to the incubation of the feeder layer produced no change in CFU-C growth. Incubation of the target cell suspension with interferon in the above-stated amounts (1 hr, 37C) before placing it in agar produced no change in CFU-C growth. The addition of interferon to the over-layer did result in a dose-dependent reduction in CFU-C growth in 4 independent experiments. These results permit the following conclusions to be drawn concerning the effect of interferon: 1) interferon does not inhibit CSF production in this system, 2) interferon is not toxic for CFU-C when incubated with it for short periods, 3) interferon does inhibit clonal proliferation of CFU-C. (no Refs) (In GER)

9. EXPRESSION OF COLONY STIMULATING ACTIVITY (CSA) AND PRODUCTION OF COLONY FORMING UNIT (S) (CFU-C) BY ACUTE MYELOID LEUKAEMIA CELLS SHOWING MATURATION IN SHORT-TERM CULTURE.

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Haematologica (Pavia); 67(2):188-198 1982

Relationship of colony stimulating activity (CSA) and colony forming unit/culture (CFU-c) to phenotypic features of the in vitro maturation process was studied in three cell populations taken directly from patients with hematologically proven acute myelocytic leukemia. Patients had no prior chemotherapy and had greater than 90% definite blast cells in peripheral blood. Relevant clinical data of the patients are presented. Cell cultures of the three patients showed almost identical growth patterns. After an initial decrease during the first 2-3 days, the number of cells increased seven-fold in the following wk, and progressively declined thereafter. Adherent cells from two patients behaved like and had the appearance of macrophages. The ability of non-adherent cells to undergo maturation in culture was confirmed by the Fc receptors on the cell surface and the presence of esterases characterizing the differentiation of monocytes or granulocytes. In two patients, cells had a high proportion of CFU-c at the beginning of the culture period (40/2 x 10(5) and 24/2 x 10(5) cells, respectively). The number of CFU-c decreased to a much lower level in both patients by day 10, followed by a secondary increase, more pronounced in one patient. Measurable levels of CSA were produced by all three non-adherent cell populations. However, the greatest activity was produced by the isolated adherent cell population of the two patients 14 days after culture and near the time of the secondary increase in CFU-c. In cell populations unable to produce phenotypic changes characteristic of maturation, both CFU-c and CSA were absent. (20 Refs)

10. VARYING EFFECTS OF SUBSTRATE AND GROWTH FACTORS IN THE CULTURE OF HUMAN-DERIVED GLIOMA, NEUROFIBROMA, AND FIBROBLAST LINES (MEETING ABSTRACT).

Kornblith PL, Hewkins C, Chronwall BM, Pollock LA
SNB, NINCDS, National Institute of Health, Bethesda, MD, 20205
In Vitro; 18(3,part2):307 1982

In an effort to enhance glial cell growth in culture, nine human glioma-derived cell lines, one human fibroblast line, and three neurofibroma-derived cell lines have been studied for the influence of collagen and fibronectin (FN) substrates as well as the effects of epidermal growth factor (EGF) and fibroblast growth factor. One additional glial tumor was explanted directly on a fibronectin substrate. Overall, growth on a fibronectin (FN) substrate was the most effective in increasing cell numbers. This was true for 7 of 9 glioma derived cell lines (increased growth ranged from 2 to 4 control). Two lines, both glioblastomas, showed growth inhibition. FN also increased

growth in one neurofibroma line, but not the fibroblast line. As a substrate for tumor explants, FN resulted in more rapid outgrowth. Collagen was relatively less effective as a substrate for the glioma lines with only three glioblastoma derived lines showing doubling or more of cell growth. One NF line showed a four-fold growth increase over control. EGF and FGF were generally of modest effect, although in one glioblastoma line that showed growth inhibition with FN, both EGF and FGF caused 2-3 fold increases. (no Refs)

11. PROPAGATION OF MOUSE CYTOTOXIC CLONES WITH CHARACTERISTICS OF NATURAL KILLER (NK) CELLS.

Kedar E, Ikejiri BL, Sredni B, Bonavida B, Herberman RB
Lab. Immunodiagnosis, NCI, Bethesda, MD, 20205
Cell Immunol; 69(2):305-329 1982

Splenocytes obtained from normal mice (BALB/c nude, BALB/c, C3H, C57Bl/6) and from mice bearing lung or pulmonary carcinomas were propagated for 1-12 mo in the presence of crude or mitogen-depleted T-cell growth factor (TCGF). Clones from several TCGF-propagated lymphoid cell lines were established by limiting dilution or the soft agar techniques. All the cultured lines and the majority of the clonal populations derived from them exhibited strong cytotoxic activity in vitro (51Cr release assay) toward a variety of syngeneic and allogeneic tumor target cells, both freshly obtained and passaged in culture, and both lymphoid and solid in origin, and including targets usually resistant to fresh NK cells. Considerable cytotoxic activity was also observed with several rat and human cultured tumor lines. Only low cytotoxic activity was detected against normal lymphoid mouse cells. Cloned populations generally exhibited more restricted target cytotoxicity than the parental cultured lines, and the pattern of reactivity varied among the clones. Of the clones tested for surface markers, all were positive for Thy 1.2, T200, and asialo GM1 and had strong binding to peanut agglutinin, all had undetectable receptors for IgG or IgM, and some were positive for Lyt 2. The cytotoxic activity was augmented by pretreatment of the effector cells with interferon and inhibited by the presence of mannose or galactose during the assay. Several clones were capable of mediating antibody-dependent cellular cytotoxicity and lectin-induced cellular cytotoxicity, and produced relatively large quantities of interferon and lymphotoxin-like material. The findings indicated continuous culturing in TCGF of previously antigen-nonstimulated mouse lymphocytes selects for the growth of at least two distinct populations with activated NK activity, one reacting preferentially with lymphoid tumor target cells, and the second reacting effectively with both lymphoid and solid tumor targets. Both populations have several features of both T lymphocytes and NK cells. (Author abstract) (48 Refs)

12. OPPOSING EFFECTS OF HUMAN PERIPHERAL BLOOD LYMPHOCYTES ON THE GROWTH OF CULTURED LEUKEMIA CELL LINES.

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Dept. Pathology, LDS Hosp., 325 8th Ave., Salt Lake City, UT, 84143
Cell Immunol; 69(2):381-387 1982

Natural killing of two human leukemia cell lines (K562 and Molt 4) in a soft-agar, clonogenic assay was shown to be the result of two opposite yet concurrent processes: target cell colony stimulation and inhibition. The stimulatory effect was demonstrable when the effector lymphocytes and target cells were separated in contiguous agar layers, suggesting mediation by a soluble factor. Similarly, stimulation occurred when the effector lymphocytes and target cells were combined at low effector-target cell ratios that do not favor direct cell contact. Target colony inhibition was found to be dominant when large E:T ratios were employed. Both target-effector binding and natural killing were significantly reduced in medium devoid of divalent cations. (Author abstract) (10 Refs)

13. RECEPTOR BINDING AND PROCESSING OF EPIDERMAL GROWTH FACTOR BY HUMAN BREAST CANCER CELLS.

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J Clin Endocrinol Metab; 55(1):86-93 1982

Epidermal growth factor (EGF) stimulates the growth of human breast cancer cells (MCF-7). To better define the mechanisms for these effects, we studied receptor binding and subsequent processing of [125I]EGF in cells growing in monolayer culture under conditions in which an effect on growth is observed. Binding was temperature dependent and was higher at reduced temperature. At 37 C, binding rapidly reached a maximum (20-60 min) and then gradually decreased. Binding was slower at a reduced temperature. High affinity was demonstrated by the ability of unlabeled EGF to compete for [125I]EGF binding; half maximal displacement was observed at an EGF concentration that also resulted in half-maximal biological activity (1-2 ng/ml). Scatchard plots derived from these data or from those of studies using increasing concentrations of [125I]EGF were curvilinear and superimposable. Plots from binding data obtained at 0 C were also curvilinear, suggesting that the processing and degradation of tracer alone cannot explain this finding. Other peptides did not compete for EGF binding. During a 6-hr incubation, progressive degradation of [125I]EGF occurred on and/or in the cells, with prompt release of low mol wt fragments into the medium. Furthermore, during this time, the bound intact EGF became more resistant to dissociation by excess unlabeled EGF. Reduced binding (50%), not due to simple occupancy, was also noted during a 2-hr preincubation with unlabeled EGF, indicating down-regulation of receptor. These data suggest the following sequence of events. At 37 C, EGF rapidly binds to specific receptors on MCF-7 cells. The bound EGF becomes more and more difficult to dissociate from the cell, and the number of available receptors is reduced, implying sequestration in the cell membrane and/or internalization of the hormone-receptor complex. Gradually, EGF is degraded, and the products are released from the cell. Human breast cancer appears to be a target for EGF action. (Author abstract) (24 Refs)

14. EFFECT OF EPIDERMAL GROWTH FACTOR ON CLONING ABILITY OF PRIMARY HUMAN TUMOR CELLS GROWN IN SEMISOLID MEDIA (MEETING ABSTRACT).

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In Vitro; 18(3,part2):307 1982

The effect of EGF (epidermal growth factor) on the soft agar colony forming ability of fresh human tumor cells was assessed in forty specimens obtained from various types of tumors such as breast, endometrium, ovary, etc. Cells from four established human tumor cell lines were also included in this study. The results showed that the addition of EGF at a concentration of 50 ng/ml resulted in a higher cloning efficien-

cy in soft agar in only 20% of the samples of fresh human tumors. In contrast, the number of colonies formed by cells from all four tumor cell lines was at least two-fold higher in semisolid medium containing EGF. Cells from fresh tumor biopsies were assayed for EGF receptors to determine if their lack of proliferative response when grown in EGF supplemented semisolid medium was due to a corresponding lack of (EGF) receptors. Specific receptors for EGF were detected by using radioiodinated EGF in early passage cell cultures from tumors of different histological types. The number of receptors ranged from 0.3 to 3.27 x 10(5) per cell. It was observed that there was not correlation between the number of EGF receptors on a cell surface and the mitogenic effect of EGF on the same cells grown in semisolid medium. (no Refs)

15. GROWTH FACTORS FOR CULTURED MAMMARY AND FIBROBLASTIC CELLS (MEETING ABSTRACT)

Rudland PS, Smith JA
Ludwig Institute, Royal Marsden Hospital, Sutton, Surrey, England
Twenty-third Annual General Meeting of the British Association for Cancer Research, March 29-31, 1982, Edinburgh, Scotland. British Association for Cancer Research 78 pp., 1982.

The growth of the mammary gland is controlled by a series of interacting mammotrophic hormones. However these hormones are relatively inactive compared with serum or novel, semi-purified growth factors (GF) in stimulating cell proliferation of cultured rat mammary gland epithelial cells. These GF's are of two types, a pituitary GF exerting systemic control and GF's from surrounding cells which may exert local control of mammary epithelial cell proliferation. The effects of the GF's can be amplified by certain mammotrophic hormones (eg insulin: I and hydrocortisone: HC). As in the mammary gland, GF's such as FGF, EGF, PGF2 alpha (fibroblast growth factor, epidermal growth factor, prostaglandin F2alpha) can stimulate DNA synthesis in quiescent 3T3 fibroblasts and this can be modified with I and HC. To explain the kinetics obtained with PGF2 alpha and FGF two operational signals are postulated, signal (1) initiates a lag phase and signal (2) determines the magnitude of the rate of cellular entry into the S phase of the cell cycle. Usually the rate of entry into S governs the rate of cell division, but when serum is completely removed additional components (eg transferrin) are required. During the lag phase the pathways generated by signal (1) and signal (2) have to interact causing increases in hypothetical protein(s) inside the cell. One of the altered properties of virally transformed cells is their lack of response to GF's. In the two cases examined by other groups the viral transforming proteins directly act on the putative intracellular pathways leading to increased cell proliferation rates. (no Refs)

16. HUMAN TERM PLACENTA CONTAINS TRANSFORMING GROWTH FACTORS.

Stromberg K, Pigott DA, Ranchalis JE, Twardzik DR
Lab. Viral Carcinogenesis, NCI, Frederick, MD, 21701
Biochem Biophys Res Commun; 106(2):354-361 1982

The isolation of three transforming growth factors (TGFs) of mol wt 6,000, 10,000 and 20,000 daltons from normal human placenta is reported. Each of these activities had characteristic properties of TGFs in that each stimulated anchorage-independent growth of normal rat fibroblasts and each competed with 125I-epidermal growth factor for receptor binding to epidermal growth factor (EGF) receptor rich human carcinoma cells. Each fraction scored in the radioreceptor assay in proportion to its normal rat kidney growth-promoting activity in soft agar. Although originally suspected to be EGF, the 6,000 mol wt fraction was shown to be a different polypeptide. Each of the four TGFs required intact disulfide bonds and withstood exposure to urea and repeated lyophilization. A several fold increase in activity was observed when the 6,000 mol wt fraction was treated with trypsin. Papain treatment, however, did strongly inhibit soft agar-stimulating activity. This system, utilizing readily available human placental tissues can be valuable in studying the progression from normalcy toward hydatidiform mole and choriocarcinoma, and the action of TGFs in stimulating this change. (19 Refs)

17. FIBROBLAST GROWTH FACTOR STIMULATES ANCHORAGE INDEPENDENT GROWTH IN AGAR OF BHK21/13 AND SV3T3 CELLS.

Zumbe A, Burk RR
Nestle Res. Lab., 1814 La Tour-de-Peilz, Switzerland
Cell Biol Int Rep; 6(5):503-511 1982

Growth of secondary baby hamster kidney fibroblasts, the partially transformed cell line BHK21/13, and the virally transformed derivatives SV3T3 and SV28 in soft agar was studied, and the response to fibroblast growth factor (FGF) was assessed. Only BHK21/13 cells responded to FGF in soft agar. Secondary hamster baby hamster kidney fibroblasts in suspension did not grow, even with FGF; SV28 cells grew independently of FGF. Balb/c 3T3 cells behaved like the secondary baby hamster kidney cells, and SV3T3 like BHK21/13, suggesting three degrees of transformation. (17 Refs)

18. IN VITRO GROWTH PROMOTION IN HUMAN MALIGNANT MELANOMA CELLS BY FIBROBLAST GROWTH FACTOR.

Richmond A, Lawson DH, Nixon DW, Stevens JS, Chawla RK
Clinical Res. Facility, Dept. Medicine, Emory Univ. Sch. Medicine, Atlanta, GA, 30322
Cancer Res; 42(8):3175-3180 1982

Bovine pituitary fibroblast growth factor (FGF) stimulates the incorporation of [3H]thymidine into DNA in serum-depleted cultures of some but not other human melanoma cells. The melanotic malignant melanoma cell line MIRW exhibited a 40% increase in [3H]thymidine incorporation into DNA and a 48% increase in cell number in response to 3.73 x 10(-9) M FGF. This same concentration of FGF produced a 22% increase in [3H]thymidine incorporation in the melanotic melanoma cell line Hs0294. However, FGF had no effect on the amelanotic melanoma cell line Hs0675, early-passage cultures of a human amelanotic melanoma (W-1), or early-passage cultures of a congenital nevus (N-1). (Author abstract) (39 Refs)

19. ENDOCYTOSIS OF NERVE GROWTH FACTOR BY PC12 CELLS STUDIED BY QUANTITATIVE ULTRASTRUCTURAL AUTORADIOGRAPHY.

Hogue-Angeletti R, Stieber A, Gonatas NK
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Brain Res; 24(1):145-156 1982

Ultrastructural quantitative autoradiography was used to investigate endocytosis of 125I-nerve growth factor (NGF) by cultured rat pheochromocytoma cell line PC12. Cells not previously exposed to NGF were incubated at 37 C with 125I-NGF for 2, 6, or 26 hr. No outgrowth of neurites have been seen under such conditions. At 2 hr, the plasma membrane was heavily labeled; at 6 and 26 hr, less but significant amounts of labeling was observed. Endocytosis of 125I-NGF was observed

at all time periods. 125I-NGF was initially associated with the plasma membrane and underwent endocytosis very slowly. Distinct endocytosis had occurred after incubation for 6 hr, but grains were still present at the plasma membrane and the nuclei were not labeled. At 26 hr, intracytoplasmic grains appeared in clusters over dense round bodies, representing secondary lysosomes. The number of grains over the plasma membrane, the Golgi apparatus, and the smooth endoplasmic reticulum did not change significantly between 6 and 26 hr. The pathway of endocytosis of 125I-NGF apparently included vesicles of the smooth endoplasmic reticulum, but not the Golgi apparatus. These findings are consistent with the hypothesis that NGF constantly occupies sites on or adjacent to the plasma membrane, and that it slowly undergoes endocytosis into lysosomes. (33 Refs)

20. CLOTTING FACTORS (THROMBIN, FACTOR XIII, FIBRONECTIN) AS REGULATORS OF FIBROBLAST, ENDOTHELIAL CELL AND TUMOR CELL PROLIFERATION (MEETING ABSTRACT).

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Onkologie; 4(5):248 1981

Thrombin and factor XIII stimulate fibroblast proliferation during a simultaneous intracellular increase in cyclic guanosine monophosphate; fibronectin on the other hand inhibits the relative 3H-thymidine uptake of fibroblasts. More recent research has been able to demonstrate that vascular wall endothelial cells are stimulated in a similar fashion by thrombin and factor XIII. Comparison studies with tumor cells (HeLa, sarcoma cells) indicate that at least in some cases tumor cells are also subject to the regulations mentioned. Incubating fibroblasts with neuraminidase completely eliminates thrombin activity and factor XIII action is shifted to higher concentrations suggesting that neuraminidase either splits a fragment off a receptor or alters the receptor's conformation. (no Refs) (In GER)

21. STRUCTURAL AND FUNCTIONAL SIGNIFICANCE OF ASSOCIATION OF A LOW MOLECULAR WEIGHT CHALONE FROM JB-1 ASCITES TUMOURS WITH HIGH MOLECULAR WEIGHT FACTORS.

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Inst. Cancer Res., Radiumstationen, Norrebrogade 44, DK-8000 Aarhus C, Denmark
Cell Tissue Kinet; 15(4):457-468 1982

The cell-specific inhibitory (chalone) activity of JB-1 ascites tumor cell proliferation has been purified using five different procedures. By combining (1) mol wt estimations based on ultrafiltration and gel chromatography, and (2) partitioning in organic solvent and ion exchangers, it is concluded that the active factor associates, in a complex manner, with various other components involving both hydrophobic and ionic forces. The active factor appears to be a slightly acidic, hydrophobic peptide (mol wt 500-1,000 D). When assessing the activity in vivo, it appears to be highly dependent on associated serum factors. Thus, the chalone studied appears to interact both structurally and functionally with various associated factors which affect its physicochemical behavior and biological activity. (Author abstract) (27 Refs)

22. TOXICITY OF MULLERIAN INHIBITING SUBSTANCE TO CELLS DERIVED FROM A HUMAN ENDOMETRIAL TUMOR (MEETING ABSTRACT).

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In Vitro; 18(3,part2):323 1982

A cell line (HEC-1A) derived from a human endometrial tumor is responsive to the presence of Mullerian Inhibiting Substance (MIS) when grown in McCoy's medium in the absence of serum but with the addition of insulin (5 ug/ml), transferrin (5 ug/ml), hydrocortisone (50 nM), EGF (epidermal growth factor) (10 ng/ml) and FGF (fibroblast growth factor) (50 ng/ml). These cells exhibit a 40% reduction in the incorporation of 3H-TDN within 12 h following the addition of MIS to the cultures when compared to similar cultures without MIS. Cells maintained in McCoy's medium with 15% fetal bovine serum show no response to MIS. Other experiments indicate that serum does not inhibit the action of MIS. MIS, isolated for these experiments from neonatal calf Sertoli cells, is a testicular glycoprotein which causes the involution of the Mullerian duct during development of the male fetus. Because the Mullerian duct is the anlage of the uterus and uterine tubes in mammals, it is postulated that cells derived from tumors of Mullerian duct origin must reacquire a sensitivity to the action of MIS by presence of fetal antigens on their cell surface. Additionally, serum must inhibit either the formation or expression of these antigens. (no Refs)

HORMONES/ STEROIDS/ PROSTAGLANDINS AS GROWTH EFFECTORS

23. BOTH TUMOR PROMOTERS AND PROSTAGLANDINS STIMULATE DNA SYNTHESIS IN PRIMARY HEPATOCYTES INCUBATED IN CALCIUM-DEFICIENT MEDIUM (MEETING ABSTRACT).

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In Vitro; 18(3,part2):302 1982

Reportedly, prostaglandin (PG) synthesis is elevated in cells exposed to tumor promoters, some of which PGs may even structurally resemble. In an investigation of the role of FGs in the promotion of an initiated liver neoplastic process we used primary cultures in neonatal rat hepatocytes grown in a medium containing either a normal or a very low calcium (Ca) complement. Thus, within a wide concentration range two established promoters, namely TPA (12-O-tetradecanoyl-phorbol-13-acetate) and phenobarbital, and the presumptive one melittin both in normal and low Ca medium were equally capable of nearly doubling the quota of 4-day-old hepatocytes flowing into DNA synthesis within the first 24 hours of treatment. Likewise, three PGs, namely A1, E1 and E2, administered at 10(-9) M enlarged by a factor of 1.8 the size of the 24-h fraction of DNA-synthesizing hepatocytes irrespectively of their incubation either in normal or low extracellular Ca. These results show that tumor promoters can promptly disconnect DNA synthesis from the physiological Ca-dependent control system. (1 Ref)

24. MECHANISM OF STIMULATION BY HUMAN INTERFERON OF PROSTAGLANDIN SYNTHESIS IN HUMAN CELL LINES.

Fuse A, Mahmud I, Kuwata T
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Cancer Res; 42(8):3209-3214 1982

Human interferon beta (IFN-beta) stimulated the synthesis of prostaglandin E (PGE) and prostaglandin F2alpha in IFN-sensitive RSa and GM258 cell lines, but not in IFN-resistant HEC-1 cells. IFN-beta at a concentration of 1,000 units/ml elicited two- to threr fold increases in PGE production in these cell lines. In the presence of exogenous arachidonic acid (1 ug/ml), IFN-pretreated cells produced five-fold more PGE compared to the cell cultures in the absence of arachidonic acid. Prednisolone, an inhibitor of phospholipase A2, at a concentration of 2 ug/ml inhibited the enhanced synthesis of PGE by IFN-pretreated cells. Indomethacin (4 ug/ml), a potent fatty acid cyclooxygenase inhibitor, also inhibited the increased synthesis of PGE. IFN stimulated the release of [14C]arachidonic acid from phospholipids but did not stimulate the activity of fatty acid cyclooxygenase. These data suggest that IFN stimulates prostaglandin synthesis by promoting the release of arachidonic acid from phospholipids. Since cycloheximide and actinomycin D inhibited the stimulation of PGE synthesis, the stimulation of prostaglandin synthesis by IFN seemed to be due to de novo enzyme synthesis, which catalyzes the release of fatty acid. Addition of exogenous PGE suppressed the growth of RSa and GM258 cells. Prednisolone and iodomethacin partially inhibited anti-cell growth activity of IFN, suggesting a possibility that IFN-inhibited cell growth was partly mediated by prostaglandins. (Author abstract) (55 Refs)

25. THE EFFECT OF CHEMICALLY DEFINED SERUM-FREE MEDIUM ON CELL LINES OF HUMAN RENAL CANCER: PROPAGATION. GROWTH REQUIREMENTS, AND TUMORIGENICITY (MEETING ABSTRACT).

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In Vitro; 18(3,part2):306 1982

Biochemical studies of human renal cell cancer (RCC) have been hampered by the lack of a chemically defined, serum-free medium (SFM). Herein we report the growth of two cell lines of human RCC on a form of SFM developed by Taub. Four cell lines of human RCC were studied: 786-0, 769-P, 1072-F, and Caki-1. Twelve-day growth curves were obtained for each line using: SFM, serum-containing medium (SCM), and a control medium. In addition, cells propagated on SFM were subcultured, plated on agar, and also inocuiated into athymic mice. Finally, hormonal-exclusion studies were performed with hydrocortisone, prostaglandin E1, insulin, transferrin, and triiodothyronine. Only 786-0 and Caki-1 had similar growth rates on SFM and SCM. The plating efficiency on SFM was 30-50% less than on SCM. The hormonal-exclusion studies revealed transferrin, hydrocortisone, and insulin essential factors for 786-0 and insulin tc be the only necessary factor for Caki-1. Following growth on SFM, both cell lines continued to produce colonies in agar and Caki-1 also produced tumors in athymic mice. To date, 786-0 and Caki-1 have been maintained in continuous culture on SFM for more than 7 months and have undergone 10 and 13 passages, respectively. (no Refs)

26. PROSTAGLANDIN E AUGMENTS THE EFFECT OF CHEMOTHERAPY ON B-16 MELANOMA IN VIVO.

Hofer D, Dubitsky A, Marti J, Santoro MG, Reilly P, Jaffe BM
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J Surg Res; 32(6):555-562 1982

The effectiveness of six chemotherapeutic agents (adriamycin, dopamine, 5-fluorouracil, nitrogen mustard, thioTEPA, and vincristine) on B-16 melanoma growth in vivo was compared with the effects of 16,16-dimethyl-PGE2-methyl ester, a long-acting synthetic analog of PGE2 (di-M-PGE2) and of chemotherapy plus di-M-PGE2. Although all the drugs were effective in slowing growth (adriamycin, dopamine, and 5-FU) or decreasing tumor size from baseline values (nitrogen mustard, thioTEPA, and vincristine), the addition of di-M-PGE2 augmented the growth inhibitory effects of each. Changes in tumor diameters were associated with similar changes in tumor cell numbers. In addition, the combinations of di-M-PGE2 plus nitrogen mustard and vincristine decreased the plating efficiency in a clonogenic assay to a greater extent than did either di-M-PGE2 or the cytotoxic drugs alone. Finally, this augmentation was not associated with systemic toxicity or changes in leukocyte count. (Author abstract) (16 Refs)

27. METHODS OF APPROACH TO CHARACTERIZATION OF NEW GROWTH FACTORS THAT PROMOTE ESTROGEN-RESPONSIVE MAMMARY TUMOR GROWTH (MEETING ABSTRACT).

Sirbasku DA, Iio M, Leland FE, Danielpour D
The University of Texas Medical School, Houston, TX, 77025
In Vitro; 18(3,part2):274 1982

Hormones such as estrogen, prolactin and thyroxine are involved in mammary tumor growth in vivo. Studies have characterized specific hormone receptors in mammary samples, and correlations have been made between the presence of the receptor and the growth responsiveness of the tumor. Despite the in vivo analysis of hormone requirements and the receptor characterizations, many in vitro studies show that the trophic effects seen in vivo are either not identified in vitro or are less than expected. We approached this paradox by asking whether estrogen-related growth factors may be involved in vivo. Since growth of mammalian cells requires the presence of polypeptide growth factors, it seemed possible that estrogens may influence either local or circulating levels of growth factors, and thus could indirectly influence the growth of mammary tumors in vivo. We considered three possible indirect mechanisms including an endocrine mechanism in which the level of a circulating growth factor is influenced by estrogens, and an autocrine/paracrine mechanism in which local levels of growth factors are involved. Our conclusions are that the endocrine and autocrine/paracrine mechanisms are functioning in vivo, and that estrogen responsive tumor growth requires more than one growth factor. (no Refs)

28. PROLACTIN STIMULATES THE GROWTH OF HUMAN PROSTATIC CARCINOMA CELL LINE DU-145 (MEETING ABSTRACT).

Webber MM, Wettlaufer JN
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In Vitro; 18(3,part2):323 1982

Cancer of the prostate is one of three leading causes of death in the adult male cancer patient yet little is known about its

etiology. The long-term goals of this study are to examine the role of prolactin (P) in growth, maintenance, functions and behavior of prostatic epithelium and its possible growth enhancing effects on prostatic cancer. DU-145 cells were grown in RPMI-1640 medium containing 2% fetal bovine serum. Ovine prolactin was incoporated into the culture medium in dose levels varying from 10(-8) IU/ml to 1.0 IU/ml. Medium was changed every other day. Cells were counted nine days after plating. Results show that levels as low as 10(-8) IU/ml stimulated growth which increased with concentration, and 10(-1) IU/ml induced maximal growth showing a four-fold increase in cell number over control cultures. Prolactin shows a bell-shaped dose-response curve. These findings are of considerable significance in determining the possible tumor enhancing effects of P in light of the fact that P levels increase with age in the human male. Carcinoma of the prostate is often treated with estrogens, which increase P secretion. (no Refs)

29. SOMATOSTATIN-REDUCED PROLIFERATION OF MURINE APLASTIC CARCINOMA CONDITIONED TO DIABETES.

Vuk-Pavlovic S, Bozikov V, Pavelic K
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Int J Cancer; 29(6):683-686 1982

Murine mammary aplastic carcinoma, when it is passed from one alloxan-diabetic animal to another, after several passages becomes conditioned to hypoinsulinemia by secreting its own substance(s) immunologically cross-reactive with insulin (SICRI). Although not cytotoxic, the total daily dose of 2.5 mg of synthetic linear somatostatin per 1 kg body mass, administered in three or more portions, retards tumor growth in normoinsulinemic mice and the proliferation of tumors preconditioned by three serial passages in diabetics. After the first passage, somatostatin treatment has no effect on the already slow growth of the unconditioned tumor. Somatostatin-reduced tumor proliferation is accompanied by the strong suppression of insulin and SICRI levels, respectively; this effect is completely abolished by the administration of modest doses of crystalline insulin. It is concluded that somatostatin retards tumor growth by diminution of plasma levels of insulin and SICRI, respectively. (Author abstract) (13 Refs)

30. EFFECT OF HYDROCORTISONE ON COLONY-FORMATION AND DIFFERENTIATION OF HEMATOPOIETIC STEM CELLS (CFUS) IN INTACT AND IRRADIATED MICE.

Shvets VN
No affiliation given
Radiobiologiia; 22(2):264-268 1982

Effect of hydrocortisone (HC) on hematopoietic stem cells was studied in (CBA x C57Bl)F1 mice. The irradiated (1,000 rads) recipients were inoculated iv with syngeneic spleen or bone marrow cells preincubated with HC (in vitro experiment). In in vivo experiment, HC was inoculated within different time periods after transplantation of bone marrow cells. In vitro incubation with HC did not affect the number and differentiation of colony-forming units (CFU) in the spleen and bone marrow. In vivo HC administration produced marked decrease of granulocytic and megakaryocytic colonies. (10 Refs) (In RUS)

31. GLUCOCORTICOIDS AND MELANOMA: RECEPTOR PROPERTIES OF DEXAMETHASONE SENSITIVE AND RESISTANT TUMORS.

Hawkins EF, Hutchens TW, Fligiel S, Horn D, Markland FS
Univ. Southern California Cancer Center, Building 1, 1720 Zonal Ave., Los Angeles, CA, 90033
J Steroid Biochem; 16(5):673-681 1982

Solid tumors were grown in hamsters with the use of dexamethasone (DEX)-sensitive (clone 6) and -resistant (clone 5) cells cloned from RPMI 3460 Syrian hamster melanoma. Studies are reported on the comparative characteristics of these tumors including their in vivo growth responses to DEX, their histology and some physicochemical properties of their cytosolic glucocorticoid receptors. Growth of tumors from DEX-sensitive cells was retarded by daily injection of DEX into the animals. Both tumor types contained cytosolic glucocorticoid receptors (which were significantly higher in clone 5 tumors), and had similar affinity and steroid specificity characteristics which enable them to exist as stable, activated (nuclear binding) complexes. Some physicochemical differences were found. Sucrose gradient analysis indicated cytosols from both tumors contained 7S receptor complexes but clone 6 contained an additional 13S form. Activated receptors isolated by diethylaminoethanol (DEAE)-cellulose chromatography from both clone 5 and 6 tumor cytosols sedimented as a single peak at 4-5S. The DEAE-cellulose profiles indicate that only the clone 6 activated complex (Peak I) was heterogeneous with respect to charge. DNA-cellulose chromatography indicated that activated receptor from clone 5 tumor cytosols may bind more tightly to DNA than those from clone 6. (21 Refs)

32. GROWTH OF NORMAL HEMOPOIETIC CELLS IN CULTURES OF BONE MARROW FROM LEUKEMIC MICE.

Hays EF, Hale L
Dept. Medicine, Sch. Medicine, Univ. California Los Angeles, 405 Hilgard Ave., Los Angeles, CA, 90024
Eur J Cancer Clin Oncol; 18(4):413-418 1982

Bone marrow from mice with spontaneous leukemia was studied in long-term culture in the presence of 10(-7) M hydrocortisone. These conditions resulted in a complete loss of leukemia cells from marrows which initially showed 95% or greater replacement with lymphoblasts. The culture conditions were also found to favor the growth of hemopoietic stem cells and the surviving cultured cells produced sufficient numbers of these cells to protect lethally irradiated (950 rads) syngeneic mice. The irradiated recipients of the cultured marrow did not develop leukemia during the 180 days of observation post-irradiation, indicating the absence of leukemia cells in the inocula. A loss of leukemia cells in vitro occurred in cultures with and without the addition of hydrocortisone to the medium. Hydrocortisone was shown to have a stimulating effect on maintenance of hemopoietic stem cells, granulocyte progenitors and granulopoiesis in these cultures. (Author abstract) (12 Refs)

CYCLIC NUCLEOTIDE/ POLYAMINES/ NUCLEOTIDES AS GROWTH EFFECTORS

33. POLYAMINES IN MAMMALIAN TUMORS. PART II.

Scalabrino G, Ferioli ME
C.N.R. Centre Res. Cell Pathology, Univ. Milan, 20133 Milan, Italy
Adv Cancer Res; 36:1-102 1982

The role of polyamines in oncology is reviewed. Polyamine concentrations in different lines of cultured neoplastic cells in response to microenvironmental conditions, in relation to growth rate and cell cycle phase, and in relation to cyclic nucleotides, and infection with nononcogenic viruses are described. The patterns of polyamines in human oncology are reviewed with respect to levels in blood, urine, and other physiological fluids; levels of polyamine biosynthetic decarboxylases in human neoplastic tissues; and metabolic conjugation of polyamines and antipolyamine antibodies in neoplastic and normal tissues. Diamine oxidase activity in human and experimental neoplasms and physiological and pharmacological inhibitors of polyamines are also discussed. Polyamine biosynthesis research is likely to lead to promising results because the principal characteristic of the phenotype of neoplasms is the lack, or at least an alteration, of regulation of metabolism, including that of polyamines. At present the role of polyamines in eliciting neoplastic behavior and permitting neoplastic growth is almost totally obscure. Studies of the relationship between polyamines and fibronectin may be important in explaining neoplastic behavior. (493 Refs)

34. REPLACEMENT OF NATURAL POLYAMINES BY CADAVERINE AND ITS AMINOPROPYL DERIVATIVES IN EHRLICH ASCITES CARCINOMA CELLS.

Alhonen-Hongisto L, Seppanen P, Holtta E, Janne J
Dept. Biochemistry, Univ. Helsinki, SF-00170 Helsinki 17, Finland
Biochem Biophys Res Commun; 106(2):291-297 1982

The replacement of the polyamines spermidine and putrescine by cadaverine in Ehrlich ascites carcinoma cells was found to be compatible with continued growth in these cells, although cell division was slowed and the changes typical of polyamine-depletion were expressed. Cells were polyamine-depleted by culture in the presence of 5 milliM difluoromethyl ornithine (DFMO) and 0.005 milliM cadaverine. Under such conditions the natural polyamines were almost completely replaced by cadaverine and its aminopropyl derivatives, mainly N-(3-aminopropyl)cadaverine. While the synthesis of RNA and DNA was decreased by only about 20% in polyamine-depleted, cadaverine containing cells, the incorporation of labeled leucine into protein was depressed by 50%. Tumor cells grown in the presence of DFMO and cadaverine exhibited ornithine decarboxylase activity 10 x higher than untreated cells. The activity of adenosylmethionine decarboxylase was also greatly elevated in cells exposed to DFMO and cadaverine, a phenomenon typical of polyamine-depleted cells. Cadaverine containing cells fully retained the enhanced uptake of methylglyoxal bis(guanylhydrazone), another characteristic of polyamine-depleted cells. (22 Refs)

35. POLYAMINES IN BIOLOGY AND MEDICINE.

Morris DR, Marton LJ
Dept. Biochemistry, Univ. Washington Sch. Medicine, Seattle, WA
Biochem Dis 8: 459 pp., 1981.

The biosynthesis, metabolism, and biological function of polyamines (PA) and their relationships to clinical medicine are discussed. Chapters related to PA biosynthesis and metabolism include discussions of the following topics: biosynthesis of putrescine, aminopropyl group transfers in PA biosynthesis, inhibition of the purification and characterization of spermine synthase from bovine brain by 5'-methylthioadenosine, biosynthesis and metabolism of PA in plants, control of ornithine decarboxylase activity in prokaryotic and eukaryotic cells, transcriptional induction by trophic hormones via cyclic AMP and cyclic AMP-dependent protein kinase pathway of ornithine decarboxylase (OD), amide-bond-forming reactions of PA, oxidation of PA and diamines, and turnover of PA. Chapters on the biological function of PA include consideration of interactions of PA with polynucleotides, PA and protein synthesis; in vivo studies of the roles of putrescine and spermidine in Escherichia coli, biochemistry, specificity, and biological effects of inhibitors of PA synthesis, consideration of the use of ornithine analogs, PA and the control of the cell cycle in animal cells, and potential use of animal cell mutants in PA research. Also discussed are approaches to the study of PA as cancer markers, clinical aspects of PA in nonneoplastic conditions, diamine oxidase (histaminase) activity in human disease, PA metabolism in humans, the PA-pyridoxal-phosphate paradigm, inhibition of S-adenosylmethionine decarboxylase as an approach in cancer therapeutics, and potential use of OD inhibitors as therapeutic agents. (129 Refs)

36. INHIBITION OF HEPATOMA CELL GROWTH BY ANALOGS OF ADENOSINE AND CYCLIC AMP AND THE INFLUENCE OF ENZYMES IN MAMMALIAN SERA.

Hargrove JL, Granner DK
(c/o Granner) Dept. Internal Medicine, Univ. Iowa and Veterans Admin. Medical Center, Iowa City, IA, 52240
J Cell Physiol; 111(3):232-238 1982

Toxic effects of cyclic AMP (cAMP) and N6,O6-dibutyryl cAMP (Bt2cAMP) were studied in hepatoma tissue culture (HTC) cells, whose growth is not inhibited by Bt2cAMP, and Reuber H35 cells, whose growth is reduced by several analogs of cAMP. In both cell lines, Bt2cAMP exerts a positive effect by increasing tyrosine aminotransferase content. Conditions were examined under which toxicity of cyclic nucleotides and adenosine analogs occurred or was prevented when serum type or treatment was varied. HTC and H35 cells were grown in Swim's S-77 medium containing 5% fetal calf serum and 5% bovine serum. Growth of HTC cells in spinner culture was inhibited in the presence of cAMP, 5'-AMP, or adenosine. Virtually no growth occurred when cAMP was present in medium containing serum heated for 20 min; in the absence of the cyclic nucleotide, cell numbers increased to 60,000/ml during 3 days of growth. Uridine and cytidine both prevented adenosine toxicity. The effects of isopentenyl adenosine on cell growth included inhibition of cell growth, nucleoside uptake, orthophosphate uptake, and DNA synthesis. Uptake of 2-deoxy-D-glucose and alpha-aminoisobutyric acid was not affected. Results suggested that toxicity of cAMP and Bt2cAMP in the two hepatoma lines is a nonspecific, adenosine-like effect attributable to metabolism of these compounds in the medium and within the cells. (44 Refs)

37. INDUCTION OF NEOPLASTIC CELL GROWTH BY QUIESCENT CELLS IS MEDIATED BY SERUM CONCENTRATION AND CAMP PHOSPHODIESTERASE INHIBITORS.

Bertram JS, Bertram BB, Janik P
Grace Cancer Drug Center, Roswell Park Memorial Inst., Buffalo, NY, 14263
J Cell Biochem; 18(4):315-538 1982

Studies have shown that for transformed mouse fibroblasts (C3H/101/2) and Lewis lung carcinoma model systems, the expression of transformation is conditional upon the permissive behavior of neighboring cells. Such permissive behavior could be due to a failure in intercellular communication or to a failure in signal reception or processing. For a constant plating density an increase in serum concentration was shown to cause a dose responsive decrease in N-methyl-N-nitro-N-nitrosoguanidine (MNNG)-induced transformation frequency in fibroblast cells. This increase in serum concentration produced an increase in saturation density of confluent cells. A model is developed which suggests that interference in communication between cells occurs during transformation. The passage of small growth inhibitory substances through gap junctions between contacting cells controls cell cycle phase. Autonomous growth would occur through attenuation of signal intensity. This studies have identified two possible factors which may control signal strength, ie, cell density and elevated cAMP. The model thus suggests that transformed cells are deficient in their ability to form communication channels with themselves or with adjacent normal cells. The major effect of phosphodiesterase inhibitors may be to increase the latent period between tumor injection and the appearance of tumor. Applications of the model to phosphodiesterase inhibitors and to tumor promotion are discussed. (42 Refs)

COFACTORS/ NUTRITIONAL GROWTH FACTORS

38. CELL CYCLE VARIATION IN 125I-LABELED EPIDERMAL GROWTH FACTOR BINDING IN CHEMICALLY TRANSFORMED CELLS.

Robinson RA, Branum EL, Volkenant ME, Moses HL
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Cancer Res; 42(7):2633-2638 1982

The chemically transformed AKR-MCA and C3H/MCA-58 cell lines spontaneously arrest growth in the G1 phase of the cell cycle due to nutrient deficiency when grown to saturation density in medium with 10% fetal bovine serum. An examination of 125I-labeled epidermal growth factor (EGF) binding in rapidly growing and G1-arrested cells showed that the G1-arrested chemically transformed cells also have a 10-to 20-fold reduction in the amount of 125I-labeled EGF binding relative to the same cells in the rapidly growing state. Recovery of receptor activity by addition of serum-free medium was inhibited by actinomycin D and cycloheximide, suggesting that new messenger RNA synthesis as well as increased protein synthesis is necessary for the recovery of EGF binding. A comparison of EGF binding in C3H/MCA-58 cells and the transformed parent line in the rapidly growing state showed the same approx level of receptor activity. The rapidly growing AKR-MCA cells had approx one-tenth the amount of EGF binding as did the rapidly growing nontransformed parent line. Analysis of data showed a 10-fold greater number of receptors in the AKR-2B cells relative to the AKR-MCA cells with a lesser difference in receptor affinity. The chemically transformed BP-3T3, like the other two chemically transformed lines, was also demonstrated to arrest growth spontaneously due to nutrient deficiency with an associated 100-fold decrease in EGF binding. Rapidly growing BP-3T3 cells had only slightly less 125I-labeled EGF binding than did the nontransformed parent line in the rapidly growing state. The data indicate that one mechanism for reduction of EGF binding in chemically transformed cells is the propensity of these cells to arrest growth in G1 at saturation density due to low-mol-wt nutrient deficiency, a state associated with decreased EGF binding. (25 Refs)

39. ULTRASTRUCTURAL AND MORPHOGENETIC RESPONSES TO VARYING CALCIUM CONCENTRATIONS BY HUMAN CERVICAL CARCINOMA CELLS (MEETING ABSTRACT).

Auersperg N, Swiergenga SH, Feng S
Anatomy Department, University of British Columbia, Vancouver V6T 1W5, British Columbia, Canada
In Vitro; 18(3,part2):305 1982

Cells of the cervical carinoma line C-4I have been propagated for over 100 passages in Waymouth' medium 752/1 and 10% fetal bovine serum. The cells form cohesive multilayered colonies with tonofilament bundles, desmosomes and an intercellular organization resembling stratified squamous epithelium. When crowded C-4I cultures were exposed to Williams medium E with Ca++ reduced to 0.02 mM, most cells detached and only a monolayer remained on the culture dishes. It maintained at high densities, these cells replicated in 0.02 mM Ca++ and could be selected to grow either in suspension or as monolayers. The monolayered C-4I cells, like epidermal keratinocytes grown in media with low Ca++, were rounded or flat, lacked desmosomes and had centrally displaced tonofilaments. Upon return of C-4I cells to media with standard Ca++ concentrations the morphologic charactistics of stratified squamous epithelium were rapidly restored. Squamous cells with cytoplasmic granules and a very low nucleocytoplasmic ratio appeared in greater numbers in 1.8 mM than in 0.82 mM Ca++. The responses of C-4I cells to varying extracellular Ca++ levels resemble those of normal keratinocytes and some epidermal carcinoma cells which also differentiate in high, but not in low extracellular Ca++. (no Refs)

40. A STUDY OF THE GROWTH REQUIREMENTS OF HUMAN TUMOUR CELLS IN TISSUE CULTURE.

Rowbottom LA, Whitehead RH, Roberts GP, Hughes LE
(c/o Hughes) Univ. Dept. Surgery, Welsh Natl. Sch. Medicine, Heath Park, Cardiff CF4 4XN, Wales
Aust J Exp Biol Med Sci; 59(1):91-100 1981

The effect of potential growth enhancing substances and other environmental factors on the growth of human cells in culture were examined. For all the cell lines tested the optimum pH range was 7.45-7.55. Optimum osmolality varied with different cell lines, optimum values being 240 milliosmoles/kg for melanoma cell lines and fetal fibroblasts, 260 milliosmoles/kg for adult fibroblasts and 300 milliosmoles/kg for a breast cancer cell line. A number of hormones were found to affect the growth of the cell lines examined. Insulin and hydrocortisone enhanced the growth of all the tumor cells tested, whereas 17 beta-estradiol enhanced the growth of breast cancer cells but inhibited the growth of melanoma cells and fibroblasts. Stilbestrol inhibited the growth of all the cell lines but prolactin enhanced the growth of melanoma cells and inhibited fi-

broblast cell growth. Addition of galactose to the medium stimulated the growth of all cell lines tested, whereas sucrose and fructose resulted in stimulation of tumor cell growth but had little effect on fibroblasts. Alpha-thioglycerol and beta-mercaptoethanol enhanced the growth of the tumor cells but had little effect on the growth of fibroblasts. A modified medium has been formulated which enhanced the growth of all the cell lines used when compared with McCoy's 5A medium. (Author abstract) (19 Refs)

EXOGENOUS AGENTS AFFECTING DIFFERENTIATION

41. PREVENTION AND REVERSAL BY A RETINOID OF 3,4-BENZPYRENE- AND CIGARETTE SMOKE CONDENSATE-INDUCED HYPERPLASIA AND METAPLASIA OF RODENT RESPIRATORY EPITHELIA IN ORGAN CULTURE.

Lasnitzki I, Bollag W
Strangeways Res. Lab., Worts Causeway, Cambridge, CB1 4RN, England
Cancer Treat Rep; 66(6):1375-1380 1982

The influence of an aromatic analog of vitamin A, etretinate, on the effects of 3,4-benzpyrene and cigarette smoke condensate has been investigated in fetal mouse lung and neonatal rat tracheas grown in organ culture. In both tissues, 3,4-benzpyrene as well as cigarette smoke condensate induces a striking increase of epithelial mitosis within 12-14 days of treatment. The increase is associated with a loss of secretory activity and of ciliary function. These changes persist in the absence of benzpyrene or smoke condensate in explants transferred to control medium. Treatment with etretinate alone does not affect the normal epithelial growth rate or normal differentiation. If combined with either benzpyrene or smoke condensate, the aromatic compound inhibits the increase in cell division and prevents the loss of secretory activity or ciliary function. In explants pretreated with 3,4-benzpyrene or cigarette smoke condensate, etretinate reduces the carcinogen- or smoke condensate-induced increase in mitotic activity to normal levels and restores secretory differentiation and ciliary function. The mechanism of action involved in the anticarcinogenic activity of the retinoid is discussed. (Author abstract) (32 Refs)

42. RESPONSES OF AMPHIBIAN EMBRYOS AND BLASTOMERES TO A TUMOR-PROMOTING PHORBOL ESTER.

Ellinger MS
Dept. Zoology, Southern Illinois Univ., Carbondale, IL, 62901
Cancer Res; 42(7):2804-2812 1982

Phorbol ester tumor promoters such as 12-O-tetradecanoylphorbol-13-acetate (TPA) alter the morphology and differentiated states of numerous cell types in vitro. It is likely that these molecules are teratogenic for vertebrate embryos in which cytodifferentiation, cell tissue interactions, and cellular motility are prominent. By examining embryonic effects, I thought that insights into mechanisms might be gained that would not be apparent in adult tissues. The purpose of this study was to characterize the responses of frog (Bombina orientalis) embryos and embryonic cells to TPA. Effects of 10 to 100 ng TPA per ml on whole embryos included epithelial dissociation, inhibition of muscle segment histogenesis and adhesive organ differentiation, and acceleration of cell sheet movements during gastrulation. Possibly correlated with accelerated gastrulation, TPA also induced circumferential rotations of lobopodial blebs in blastula cells cultured on plastic. This capacity is normally not seen until later developmental stages and is most prominent in areas undergoing morphogenetic movements. For blastula cells cultured on agar, TPA (1.0 to 10 ng/ml) inhibited cytokinesis but not karyokinesis, leading to the formation of abnormal multipolar spindles. A similar uncoupling of cytokinesis and karyokinesis in adult tissues could lead to mitotic aneuploidy, possibly an important step in tumor promotion. The amphibian embryo should be a useful organism for further studies on phorbol ester tumor promoters. (Author abstract) (49 Refs)

43. INHIBITION OF IODINATED NERVE GROWTH FACTOR BINDING BY THE SUSPECTED TUMOR PROMOTERS SACCHARIN AND CYCLAMATE.

Ishii DN
Dept. Pharmacology, Coll. Physicians and Surgeons, Columbia Univ., 630 West 168th St., New York, NY, 10032
JNCI; 68(2):299-303 1982

The strong correlation between tumor-promoting activity and the capacity to inhibit nerve growth factor-induced neurite outgrowth for a number of compounds prompted studies on the suspected bladder tumor promoters saccharin and cyclamate. These artificial sweeteners reversibly inhibited neurite outgrowth and binding of [125I]nerve growth factor in embryonic chick sensory ganglia cells. Curves showing the dose-dependent inhibition of response and the dose-dependent inhibition of binding to high affinity sites were closely parallel. The inhibition appeared to be due to a reduction in the affinity rather than to a change in the number of binding sites, as indicated by the extent of reduction in the association rate. These results suggest that alteration of cellular differentiation by tumor promoters may result from interactions with receptor systems that regulate specialized cell functions. (Author abstract) (41 Refs)

44. INTERFERON INHIBITS PWM INDUCED B CELL DIFFERENTIATION BUT NOT ONSET OF PROLIFERATION.

Yen A, Lewin D, Fairchild D
Dept. Physiology and Biophysics, Univ. Iowa, Basic Science Building, Iowa City, IA, 52242
Leuk Res; 6(2):221-229 1982

Results of a test, using pokeweed mitogen (PWM) driven human lymphocyte system to elicit an in vitro humoral response are reported. The net DNA synthesis by PWM stimulated cells was reduced by approx 40% in the presence of interferon (INF) after 96 and 144 hr of culture. The presence of INF resulted in reduced net synthesis of DNA, indicating reduced net cell proliferation due to INF. The distribution of cells with respect to the G1/0-, S-, and G2 + M-cell cycle phases was similar in PWM stimulated cell cultures with or without INF. INF treated cells initiated DNA synthesis at the same time as untreated cells. The presence of INF, thus, did not affect the minimum transit from G0 to S. No difference in the population size was observed for cells in the presence or absence of INF until after 48 hr. However, the size of INF treated populations thereafter was persistently less. This is consistent with the INF induced reduced proliferation. The smaller population size of INF exposed populations is attributable to the reduction in proliferation rate relative to the loss rate. The rates of PWM induced proliferation by T cells and non-T cells

were approx equal and reduced similarly in the presence of INF. The observed fraction of the population composed of T cells remained stable up to 144 hr for both cells treated and not treated with INF. The enrichment in the relative number of plasmacyte producing either kappa or lambda light chain antibody, due to PWM induced B cell differentiation, was reduced by INF. The observed reduced protein synthesis rate corroborates the reduced enrichment of immunoglobulin producing cells due to INF. (28 Refs)

OTHER RELATED STUDIES

45. TRANSLATION OF MRNA FOR HUMAN GRANULOCYTE-MACROPHAGE COLONY STIMULATING FACTOR.

Lusis AJ, Golde DW, Quon DH, Lasky LA
Div. Hematology/Oncology, Dept. Medicine, UCLA Sch. Medicine, Los Angeles, CA, 90024
Nature; 298(5869):75-77 1982

Translation and partial characterization of a messenger RNA (mRNA) for human granulocyte-macrophage colony stimulating factors (GM-CSF) that stimulates both granulocyte and macrophage colonies are reported. The mRNA was isolated from human T-lymphocyte cell line, and when injected into Xenopus laevis oocytes it directed the synthesis of biologically active GM-CSF. The GM-CSF translated into oocytes also stimulated the growth in agar of a human myeloid leukemia cell line KG-1. As in the case of bone marrow cells, medium from oocytes injected with water or control RNA had no effect on the growth of KG-1 cells. However, homogenates of both injected and uninjected oocytes significantly inhibited cell growth. (21 Refs)

46. EFFECTS OF PHORBOL MYRISTATE ACETATE AND A LYMPHOKINE ON CYCLIC 3':5'-GUANOSINE MONOPHOSPHATE LEVELS AND PROLIFERATION OF MACROPHAGES.

Hadden EM, Sadlik JR, Coffey RG, Hadden JW
Dept. Pharmacology, Univ. South Florida Medical Center, Tampa, FL, 66312
Cancer Res; 42(8):3064-3069 1982

The tumor promoter phorbol myristate acetate (PMA) was compared to a lymphokine macrophage mitogenic factor (MMF) for its ability to induce replication of guinea pig peritoneal and alveolar macrophages. Like MMF, PMA induces DNA synthesis of both cell populations with peak thymidine incorporation at 72 hr of culture. Optimal concentrations of PMA for the peritoneal and alveolar cells were 1.6 x 10(-7) and 1.6 x 10(-9) M, respectively. the magnitude of the effect is slightly less than MMF but greater than that of phytohemagglutinin or concanavalin A. Indomethacin added to inhibit prostaglandin synthesis potentiates the effects of MMF but has little effect on the actions of PMA and the other mitogens. Potentiation by indomethacin of the effects of PMA on the peritoneal cell was observed only at the suboptimal concentration of PMA (1.6 x 10(-8) M). By adherence criteria and density gradient fractionation, the cell responding to PMA is confirmed to be the macrophage. Cell counts and nuclear radioautography confirm that replication in this system is reasonably well reflected by thymidine incorporation. The effects of PMA and its analogs as macrophage mitogens correlate with their tumor-promoting effects. Both PMA and MMF induce early increases in peritoneal macrophage levels of cyclic 3':5'-guanosine monophosphate without changes in the levels of cyclic 3':5'-adenosine monophosphate. These studies indicate that PMA offers a useful probe of macrophage function. (Author abstract) (41 Refs)

47. PHORBOL DIESTER AND EPIDERMAL GROWTH FACTOR RECEPTORS IN 12-O-TETRADECANOYLPHORBOL-13-ACETATE-RESISTANT AND -SENSITIVE MOUSE EPIDERMAL CELLS.

Colburn NH, Gindhart TD, Hegamyer GA, Blumberg PM, Delclos KB, Magun BE, Lockyer J
Lab. Viral Carcinogenesis, NCI, Frederick, MD, 21701
Cancer Res; 42(8):3093-3097 1982

Several cell variants have been isolated from promotable mouse JB6 epidermal cells which are resistant either to mitogenic stimulation at quiescence or to promotion of anchorage independence by 12-O-tetradecanoylphorbol-13-acetate (TPA). Such resistant variants would be expected to lack one or more steps in the TPA response pathway leading to mitogenesis or promotion of tumor cell phenotype. This report is concerned with determining whether resistance is attributable to lack of receptors for phorbol diesters or epidermal growth factor (EGF, a potential mediator) or to absence of receptor down modulation following ligand binding. The results show that neither lack of phorbol diester receptors nor absence of down modulation can be demonstrated in the TPA-resistant variants. The phorbol ester binding affinity is also not altered in the resistant variants. The presence of EGF receptors cannot be an absolute requirement for TPA promotion sensitivity since three of the TPA-promotable cell lines lack available EGF receptors. Lack of EGF receptors may account for TPA mitogen resistance in at least three of four resistant variants. The TPA-induced EGF binding decrease occurs in both sensitive and resistant variants. Thus, phorbol diester binding and receptor down modulation remain as possible required events in mitogenic and promotion responses to TPA. EGF receptors are clearly not necessary for TPA promotion of anchorage independence in JB6 cells but may mediate mitogenic stimulation of these cells by TPA. (Author abstract) (31 Refs)

48. CARCINOGENESIS AND BIOLOGICAL EFFECTS OF TUMOR PROMOTERS.

Hecker E
Inst. Biochemistry, German Cancer Res. Center, Heidelberg, W. Germany
Carcinogenesis 7: 664 pp., 1982.

The most recent experimental findings on the etiological, cell biological, and molecular facets of carcinogenesis are reviewed. The basic problems of whether specific carcinogens function as promoters of carcinogenesis, as stimulators of initiation, or both, are addressed with respect to in vivo animal studies, epidemiological studies, and in vitro studies of tumor promotion. Carcinogenesis and tumor promotion in vivo is discussed with respect to the skin as a target tissue and nonepidermal target tissues. Effects of tumor promoters on cell proliferation and differentiation are reviewed for the epidermis and nonepidermal cell systems. Membrane interactions and receptors, effects on intercellular communication and cell metabolism, and viral and immunologic aspects are reviewed. Epidemiologic studies of esophageal cancer in Linxian County, China, nutritional carcinogenesis, and breast cancer as an example of promoting effects are discussed. (1368 Refs)

49. THE EFFECTS OF TUMOUR PROMOTERS ON THE MULTIPLICATION AND MORPHOLOGY OF CULTURED HUMAN EPIDERMAL KERATINOCYTES.

Parkinson EK, Emmerson A
Dept. Cancer Studies, Medical Sch., Univ. Birmingham, Birmingham B15 2TJ, England
Carcinogenesis; 3(5):525-531 1982

The tumor promoter phorbol,12-myristate,13-acetate (PMA) was shown to inhibit the multiplication of five strains of human epidermal keratinocytes, co-cultivated with 3T3 feeders in the presence of hydrocortisone, cholera toxin and epidermal growth factor. The effect was dose dependent between 10(-9) M and 10(-8) M and this dose response was not affected by omission of any of the tissue culture additives. Experiments performed in 3T3-conditioned medium demonstrated that PMA acted directly on the keratinocytes. Exposure to 10(-8) M PMA for greater than 12 hr resulted in a loss of keratinocyte colony-forming efficiency, followed 24-48 hr later by altered cell morphology, loss of cell to cell and cell to substratum adhesion and accelerated cytoplasmic and nuclear destruction. PMA-treated keratinocytes also became permeable to Trypan Blue, and many eventually formed cornified envelopes. Other phorbol esters tested produced similar effects to PMA in accordance with their reported tumor promoting activity, although mezerein (a weak tumor promoter) was more potent than PMA. The non-diterpene tumor promoters anthralin and phenobarbital did not have the same effects as PMA. The morphological effects were not produced in other cell types tested consistent with the specific low dose effects of PMA. (Author abstract) (43 Refs)

50. BENZO[A]PYRENE AND OTHER INDUCERS OF CYTOCHROME P1-450 INHIBIT BINDING OF EPIDERMAL GROWTH FACTOR TO CELL SURFACE RECEPTORS.

Ivanovic V, Weinstein IB
Haskell Lab., DuPont Co., P.O. Box 50, Newark, DE, 19711
Carcinogenesis; 3(5):505-510 1982

The binding of 125I-labeled epidermal growth factor (EGF) was utilized to monitor possible cell surface effects of polycyclic aromatic hydrocarbon carcinogens. Exposure of confluent C3H 10T1/2 mouse fibroblasts to 1 uM benzo[a]pyrene led to a time-dependent decrease of EGF binding. By 24 hr, EGF binding was only 5% that of control cultures. In contrast, benzo[a]pyrene-7,8-diol-9,10-oxide did not significantly alter EGF binding, indicating that the inhibition by benzo[a]pyrene was not simply due to DNA damage. A curvilinear Scatchard plot in the control cells was consistent with the presence of two classes of EGF receptors having differing affinities. Our results suggest that the major effect of benzo[a]pyrene was a reduction in receptor number rather than affinity, although other interpretations have not been excluded. Progesterone, 17beta-estradiol, benzo[e]pyrene, cholesterol, phenobarbital, 1,1-bis-(p-chlorophenyl)-2,2,2-trichloroethane, hexachlorobenzene or pregnenolone-16alpha-carbonitrile, did not inhibit EGF binding. On the other hand, several known inducers of P1-450 were very effective inhibitors of EGF binding. These included: dimethylbenz[a]anthracene, 3-methylcholanthrene, benzo[a]pyrene, benz[a]anthracene, beta-naphthoflavone and alpha-naphthoflavone. We postulate that the binding of certain polycyclic aromatic hydrocarbons to the Ah receptor may induce not only specific drug metabolizing enzymes but also inhibition of EGF binding, and possible other cell effects. Further studies are required to verify this hypothesis. (Author abstract) (41 Refs)

51. IMMUNE (GAMMA) INTERFERON PRODUCTION BY MURINE T CELL LYMPHOMAS.

Landolfo S, Arnold B, Suzan M
Istituto di Microbiologia, Univ. Torino, Via Santena 9, 10126-Torino, Italy
J Immunol; 128(6):2807-2809 1982

Various cloned murine T cell hybridomas and T cell lymphomas were evaluated for their ability to produce interferon (IFN). Two T cell tumor clones, L12-R1 and L12-R4, derived from the spontaneously in vitro transformed cell line L12 originally established from fetal calf serum-primed C57BL/6 spleen cells were found to produce high IFN amounts upon mitogen stimulation. Phorbol myristate acetate led to maximal IFN production (2,187 IU) by L12-R4 cells at concentrations of 2 x 10(-7) M, whereas concanavalin A and phytohemagglutinin induced lower levels of IFN synthesis (160 to 243 IU). None of the cell lines tested produced IFN constitutively or upon lipopolysaccharide stimulation. The IFN was characterized as immune (gamma) by being labile at pH 2 and neutralized by two rabbit anti-murine IFN-gamma antisera but not by antiserum to murine leukocyte (alpha) and fibroblast (beta) IFN. Phenotypic characterization of IFN-gamma-producing cells showed the L12 clones to be Thy-1.2+, Lyt-1+, 23-, and Ig-. The L12-R4 tumor cells therefore provide a unique source of IFN-gamma for purification, and may represent a useful model for studying the molecular mechanisms involved in T cell differentiation leading to IFN-gamma production. (Author abstract) (24 Refs)

52. CONTRIBUTIONS OF SECRETED TUMOR CELL PRODUCTS TO METASTASIS.

Roblin R
Cancer Biology Program, Frederick Cancer Res. Center, Frederick, MD
Cancer Biol Rev; 2:59-94 1981

The roles of products secreted by tumor cells whose action could play a role in the neovascularization, intravasation, capillary arrest, and extravasation stages of hematogenous metastasis are reviewed. Tumor angiogenesis factors, including the properties of epidermal growth factor and fibroblast growth factor, and hydrolytic enzymes, including plasminogen activator, collagenase, lysosomal hydrolase, and procoagulants are discussed. (148 Refs)

53. EFFECTS OF HETEROLOGOUS ANTISERA TO RAT ALPHA-FETOPROTEIN ON GROWTH OF CULTURED FETAL RAT HEPATOCYTES.

Watabe H, Tsuji Y, Saito T
Dept. Biochemistry, Faculty of Pharmaceutical Sciences, Higashi-Nippon-Gakuen Univ., Ishikari-Tobetsu, Hokkaido 061-02, Japan
Tumor Res (Sapporo); 16:29-36 1981

The effect of heterologous antisera to alphafetoprotein (AFP) on the growth of cultured fetal rat hepatocytes which produce AFP and other serum proteins was investigated. Horse anti-AFP induced marked inhibition of cell growth compared to both normal horse and fetal calf sera. Inhibition by anti-AFP horse serum 5 days after plating was 83.3%. Rabbit anti-AFP serum was inhibitory to cell growth as well, although to a much lesser degree than horse anti-AFP. Goat anti-AFP serum treatment had little effect on cultures even when the concentration of antiserum was raised to 20%. Both antisera from rabbit and goat showed non-specific recovery efficiency on hepatocyte growth. An inhibitory effect was observed even when horse anti-AFP was added 2 days after plat-

ing. Enhancement of cell growth was observed in cultures treated with normal horse serum. Rabbit anti-albumin serum was not growth inhibitory even at high concentrations. Cells treated with horse anti-AFP showed faint immunoperoxidase staining of AFP and albumin, irregularity of the cell periphery, and many dead cells adhering to the dyes. These results are consistent with cell killing induced by the antisera. (27 Refs)

54. BIOPTERIN AS AN INDICATOR OF CELL PROLIFERATION IN LEUKEMIA, SOLID TUMORS AND FOLLOWING BONE MARROW TRANSPLANTATION (MEETING ABSTRACT).

Ziegler J, Fink M, Kolb HJ, Maier K
Institut fur Biochemie, GSF, Munchen, W. Germany
Onkologie; 4(5):276 1981

Pterins have been described as indicators of intensified cell proliferation. Our studies involving experimental bone marrow-transplantation have shown that hematopoietic cell proliferation is correlated with biopterin synthesis. Its measurement in different blood cell fractions in leukemia to date has revealed a pathologic and characteristic distribution pattern for the particular leukemia in all cases. Free biopterin is rapidly eliminated by the kidneys. A pterin metabolite however is found in connection with the onset of proliferation as the chromophore group on an acidic glycoprotein whose biochemical characteristics have been determined. As of now 500 screenings of blood samples have revealed an elevation of this pterin-associated glycoprotein in approx 60% of patients with solid tumors but in less than 5% of patients with non-malignant diseases. (no Refs) (In GER)

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CELL AND SERUM DERIVED GROWTH EFFECTORS

1. PURIFICATION AND PARTIAL CHARACTERIZATION OF A LOW MOLECULAR WEIGHT ANGIOGENESIS FACTOR FROM THE WALKER 256 RAT CARCINOMA.

Watt SL
The Johns Hopkins University, Baltimore, MD
Diss Abstr Int [B]; 42(9):3673-B 1982

The purified angiogenesis factor did not possess a primary amine or a phosphate group. The factor absorbed ultraviolet light, with a maximum at 260 nm. Preliminary gas chromatography/mass spectroscopy data indicated that the factor did not volatilize at temperatures up to 300C, even after methylation and trimethylsilyl derivatization. Although preliminary, these data suggest that the factor is not a peptide or a nucleotide and that the factor might possibly be a derivative of arachidonic acid. (no Refs)

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HE 20.3173/2:

2. PRELIMINARY CHARACTERIZATION OF MALIGNANT MAMMARY CELL GROWTH INHIBITORS IN NORMAL HUMAN SERUM.

Pigott DA
The Pennsylvania State University, Univeristy Park, PA
Diss Abstr Int [B]; 42(7):2724-B 1982

Plasma-derived human serum (PDS) from normal donors inhibits the growth of the human mammary carcinoma cell line, MCF-7. The growth of HBL-100 cells, a line derived from normal mammary tissue, is not affected. Fractionation of PDS reveals three components responsible for specific inhibition of MCF-7 cell proliferation. Two additional components inhibit proliferation of both cell types. Preliminary characterization of the MCF-7 specific activities indicates the involvement of an IgG and two proteins of molecular weight 70,000 and 50,000 daltons. Non-specific activity is attributable to a lipid component and a 20,000 MW protein. Some evidence exists which indicates a possible relationship between the 70-, 50-, and 20,000 MW proteins. In addition, mitigation of the MCF-7 specific activities is demonstrated by a component(s) also present in PDS. These results indicate that malignant cell proliferation in vivo could be subject to constraints beyond the classical antibody and cell-mediated tumor-immune responses. (no Refs)

3. FACTORS MODIFYING THE ANTITUMOR EFFECT OF CHALONES FROM EHRLICH ASCITES CARCINOMA CELLS.

Kraskovskii GV, Mel'nov SB
Byelorussian Inst. Genetics and Cytology, Minsk, USSR
Dokl Akad Nauk BSSR; 26(6):562-564 1982

The effect of chalone from Ehrlich ascites carcinoma (EAC) on cell proliferation was studied in Af mice and in hyperdiploid line of EAC cells. In vitro incubation of tumor cells with EAC extract resulted in significant inhibition of EAC growth in mice. Transplantation of tumor cells without in vitro incubation with EAC extract did not inhibit proliferation of EAC cells in vivo. Administration of hydrocortisone prior to tumor transplantation produced inhibition of tumor growth. The most significant inhibition of tumor growth was observed in mice inoculated with hydrocortisone and transplanted with tumor cells that were preincubated with EAC extract in vitro. Incubation of target cells with trypsin enhanced the effect of chalone. These findings confirm the chalone activity of the extract of EAC cells. The most pronounced effect of chalone on proliferation of tumor cells was observed after in vitro incubation with target cells. (14 Refs) (In RUS)

4. MECHANISMS THAT UNCOUPLE GROWTH AND DIFFERENTIATION IN MYELOID LEUKEMIA CELLS: RESTORATION OF REQUIREMENT FOR NORMAL GROWTH-INDUCING PROTEIN WITHOUT RESTORING INDUCTION OF DIFFERENTIATION-INDUCING PROTEIN.

Lotem J, Sachs L
Dept. Genetics, Weizmann Inst. Science, Rehovot 76100, Israel
Proc Natl Acad Sci USA; 79(14,part1):4347-4351 1982

There are different macrophage- and granulocyte-inducing (MGI) proteins. Normal myeloid precursors are induced to multiply by one form (MGI-1) and to differentiate by another form (MGI-2). There are clones of myeloid leukemia cells that no longer require MGI-1 for growth but can still be induced to differentiate by MGI-2. After induction of differentiation in these leukemia cells by adding MGI-2 or inducing endogenous production of MGI-2 by lipopolysaccharide, the differentiating leukemia cells, like normal cells, again required MGI-1 for growth. This growth requirement for MGI-1 could not be substituted for by adding other protein growth factors such as epidermal, fibroblast, or nerve growth factor or insulin. Induction of differentiation in these leukemia cells by dexamethasone, arabinonucleoside (cytosine arabinoside), or methotrexate instead of by MGI-2, did not restore the requirement of MGI-1 for growth. Mutant myeloid leukemia cells that could not be induced to differentiate by MGI-2 also did not show this restoration of the requirement of MGI-1 for growth. MGI-1 in normal cells induced cell growth and also induced MGI-2, so that the cells could then differentiate by the endogenously produced MGI-2. However, MGI-1 did not induce production of MGI-2 in the leukemia cells, even though they again required MGI-1 for growth, so that there was no induction of differentiation after adding MGI-1. This lack of induction of differentiation-inducing protein by growth-inducing protein has thus identified an effective mechanism for uncoupling of growth and differentiation in malignant cells. (Author abstract) (43 Refs)

5. HIGH-DENSITY LIPOPROTEINS AND THE PROLIFERATION OF HUMAN TUMOR CELLS MAINTAINED ON EXTRACELLULAR MATRIX-COATED DISHES AND EXPOSED TO DEFINED MEDIUM.

Gospodarowicz D, Lui GM, Gonzalez R
Cancer Res. Inst., Univ. California Medical Center, San Francisco, CA, 94143
Cancer Res; 42(9):3704-3713 1982

The ability of high-density lipoprotein (HDL) to support the growth of an established tumor cell line exposed to defined medium supplemented with transferrin has been examined. Low-density A-431 carcinoma cells maintained on extracellular matrix- or fibronectin-coated dishes proliferated actively when exposed to a synthetic medium supplemented with HDL, 500 ug protein per ml. Epidermal growth factor added at concentrations above 0.5 ng/ml inhibited cell growth, while at concentrations above 5 ng/ml it was cytotoxic. Among the various substrata tested for their ability to support the active proliferation of low-density A-431 cells when exposed to transferrin and HDL, plastic was the least efficient. On fibronectin-coated dishes, cells ceased to proliferate after 8 population doublings, while on extracellular matrix-coated dishes cells could be passaged for 50 population doublings. In the case of colon carcinoma, rhabdomyosarcoma, and Ewing's sarcoma cells exposed to medium supplemented with transferrin, the addition to the cultures of HDL alone resulted in a growth rate and final cell density which were similar to those observed when cells were exposed to serum-supplemented medium. In the case of the mammary carcinoma cell lines MCF-7 and ZR-75-1, HDL also supported cell growth, although to a lesser extent than did serum. The present study therefore indicates that HDL is capable of supporting, either totally or partially, the in vitro proliferation of tumor cells. (Author abstract) (39 Refs)

6. GENERATION OF LONG-TERM T-LYMPHOID CELL LINES WITH SPECIFIC CYTOTOXIC REACTIVITY FOR A SYNGENEIC MURINE LYMPHOMA.

Eberlein TJ, Rosenstein M, Spiess PJ, Rosenberg SA
NIH, Building 10, Room 10N 116, Bethesda, MD, 20205
JNCI; 69(1):109-116 1982

The long-term in vitro growth in T-cell growth factor (TCGF) of murine cytotoxic T-lymphoid cells directed against syngeneic tumor antigens was investigated. C57BL/6 mice

were immunized to syngeneic FBL-3 lymphoma by two ip injections of irradiated FBL-3 lymphoma cells. Splenocytes from these animals were injected into mice with disseminated lethal FLB-3 tumor. The injection of cyclophosphamide (Cy) plus immunized lymphocytes significantly improved survival with cure of 53% of 38 animals. In comparison, treatment with Cy alone resulted in 0 to 31 cured and treatment with Cy plus unimmunized cells resulted in 0 of 40 cured (p less than 0.0005). These in vivo immunized lymphocytes were reexposed to FBL-3 tumor in vitro for 5 days in lectin-free TCGF (LF-TCGF). Although in vivo and in vitro sensitized lymphocytes exhibited no cytotoxicity toward fresh FBL-3 tumor cells in an 18-hour 51Cr release assay, expansion of appropriately sensitized cells in LF-TCGF resulted in significant lysis of fresh FBL-3 tumor cells. This significant lysis was specific and lysed syngeneic FBL-3 but not syngeneic MCA-103 fresh tumor targets. This maximal specific cytotoxicity was maintained for 2.5 months. A screening assay was developed that permitted rapid identification and isolation of low-frequency cytotoxic clones with reactivity specific for FBL-3 tumor. Several of these cloned cells were grown for almost 3 months with maintenance of high degrees of specific lysis (as much as 4,500 lytic U/10(6) cells). These cytotoxic lines and clones will be of value for the study of tumor-host immunologic interactions and perhaps for use in adoptive immunotherapy. (Author abstract) (68 Refs)

7. MORPHOLOGICAL AND PROLIFERATIVE CHARACTERISTICS OF HUMAN BREAST TUMOR CELLS CULTURED ON PLASTIC AND IN COLLAGEN MATRIX.

Leung CK, Shiu RP

(c/o Shiu) Dept. Physiology, Univ. Manitoba, Winnipeg, Manitoba R3E 0W3, Canada

In Vitro; 18(5):476-482 1982

Collagen, a major component of the extracellular matrix, is important in maintaining the in vivo characteristics of epidermal cells in vitro. In the present study, the morphological and proliferative characteristics of two human mammary epithelial cell lines (T-47D and MCG-7) cultured in cowhide collagen (Vitrogen 100) were studied. When grown in collagen, the tumor cells displayed a spherical shape and formed multilayered, tumorlike aggregates; desmosomes were observed between cells. In contrast, both cell lines grew as monolayers on plastic substratum; cells were characteristically flat and polygonal. When grown in collagen matrix, the human breast cancer cells became more dependent on serum for growth: cells proliferated in the presence of 10% fetal bovine serum (FBS) but failed to grow in 1% serum. On the other hand, these cells proliferated rapidly in 1% serum when they were grown on plastic. Even in 10% serum the doubling time of cells cultured in collagen was longer than that of cells maintained on plastic. In addition, cells cultured in collagen proliferated rapidly in a serum-free medium containing insulin, epidermal growth factor (EGF), estrogen, and transferrin. The collagen gel system may be useful for characterizing physiologically important trophic factors that regulate the proliferation and other functions of human breast tumor cells. (Author abstract) (19 Refs)

8. POTENTIATION OF FIBROBLAST GROWTH BY NODULAR SCLEROSING HODGKIN'S DISEASE CELL CULTURES.

Newcom SR, O'Rourke L

Section Medical Oncology, Oregon Health Sciences Univ., 3181 S.W. Sam Jackson Park Road, Portland, OR, 97201

Blood; 60(1):228-237 1982

Cell cultures were established from eight lymph nodes replaced by nodular sclerosing Hodgkin's disease. Serum-containing and serum-free conditioned media from these cultures potentiated fibroblast growth and were found to be consistently more potent than fibroblast growth factor, 100 ng/ml, every other day. Both a proliferative response and transformation-like growth were observed using BALB/c 3T3 cells, human diploid fibroblasts, and human embryonic fibroblasts as target cells. The Hodgkin's disease growth factor(s) was not produced by fibroblasts or or lymphocytes in the Hodgkin's cultures and was most potent when the Hodgkin's cultures had been enriched with Hodgkin's giant cells. Removal of normal macrophages decreased the proliferative activity but did not eliminate it or nonadherent growth of 3T3 cells in agar. Control cultures of six nonmalignant lymph nodes, a Lennert's lymphoma, a mixed cellularity Hodgkin's disease lymph node, and a malignant histiocytosis cell line suggested that among lymph node disorders, this feature may be relatively specific for nodular sclerosing Hodgkin's disease. (Author abstract) (34 Refs)

9. ISOLATION AND CHARACTERIZATION OF THE GROWTH-STIMULATING FACTOR IN SUPERNATANTS OF HERPES SIMPLEX VIRUS TYPE 2 INFECTED CELLS.

Reiss-Gutfreund RJ, Letnansky K, Desser H

Inst. Cancer Res., Borschkegasse 8a, A-1090 Vienna, Austria

Oncology; 39(5):319-324 1982

Lytic infection of CV1 cells with herpes simplex virus Type 2 does not stimulate ornithine decarboxylase activity and there is no correlation between polyamines and a growth-stimulating factor (GSF) which is present in the supernatant of the cultures. The factor was partially purified by gel filtration on Sephadex G-50 and polyacrylamide gel electrophoresis. Gel filtration as well as dialysis through membranes with different pore diameters indicated a mol wt between 3,500 and 10,000 daltons. GSF is not extracted from aqueous solutions by diethyl ether. The activity is partially sensitive to trypsin digestion and is completely destroyed by periodate oxidation. These results suggest that GSF is a glycoprotein or a glycopeptide whose mitogenic activity resides mainly in the sugar moiety. (Author abstract) (24 Refs)

10. ADOPTIVE CHEMOIMMUNOTHERAPY OF A SYNGENEIC MURINE LYMPHOMA WITH LONG-TERM LYMPHOID CELL LINES EXPANDED IN T CELL GROWTH FACTOR.

Eberlein TJ, Rosenstein M, Spiess P, Wesley R, Rosenberg SA

Surgery Branch, Div. Cancer Treatment, NCI, NIH, Bethesda, MD, 20205

Cancer Immunol Immunother; 13(1):5-13 1982

The cure of disseminated syngeneic FBL-3 lymphoma in female C57/BL/6 mice was attempted with the use of in vivo-immunized cells, in vitro-sensitized cells, whole lymphoid populations expanded in T cell growth factor (TCGF), and with cloned cytotoxic cells expanded in TCGF. In 3/3 experiments, 20/38 mice receiving cytoxan (CY) plus in vivo-immunized

lymphoid cells were cured of tumor compared to 0/31 mice treated with CY plus normal lymphocytes. In vivo-immunized lymphocytes were resensitized to irradiated FBL-3 tumor in complete medium (CM) or lectin-free TCGF (LF-TCGF). In the CM group 41/50 animals were cured. In the LF-TCGF group 36/61 animals were cured. No significant difference was observed in the in vivo effects of cells in vitro sensitized (IVS) in CM or LF-TCGF. IVS cells were resensitized in vitro to irradiated FBL-3 tumor for 5 days. Simultaneously, normal C57BL/6 lymphocytes were co-cultured with normal C57BL/6 irradiated stimulators. Neither of these groups showed significant lysis of fresh FBL-3 tumor in an 18-hr 51Cr-release assay in six of eight experiments. Treatment of mice with CY plus immune lymphocytes sensitized to normal C57BL/6 stimulators and then expanded in LF-TCGF resulted in no increase in survival compared with CY alone. Treatment of mice with CY plus immune lymphocytes resensitized to FBL-3 tumor in vitro and expanded in LF-TCGF cured 7/27 animals and prolonged survival over that in all control groups. Treatment with CY plus normal nonimmune lymphocytes resensitized to normal C57RL/6 stimulators and expanded in LF-TCGF for 2.5 mo did not prolong survival compared with CY controls. CY plus immune lymphocytes sensitized to FBL-3 tumor and expanded continuously in LF-TCGF for 2.5 mo showed marked prolongation of survival and cured 5/9 mice. Implications of results for application of these techniques to other less antigenic tumors and human cancers are discussed. (49 Refs)

11. NERVE GROWTH FACTOR ATTENUATES NEUROTOXIC EFFECTS OF TAXOL ON SPINAL CORD-GANGLION EXPLANTS FROM FETAL MICE.

Peterson ER, Crain SM
Dept. Neuroscience, Albert Einstein Coll. Medicine, Bronx, NY, 10461
Science; 217(4557):377-379 1982

Most neurons in organotypic cultures of dorsal root ganglia from 13-day-old fetal mice require high concentrations of nerve growth factor for survival during the 1st wk after explantation. These nerve growth factor-enhanced sensory neurons mature and innervate the dorsal regions of attached spinal cord tissue even after the removal of exogenous growth factor after 4 days. In cultures exposed for 4 days to nerve growth factor and taxol (a plant alkaloid that promotes the assembly of microtubules) and returned to medium without growth factor, greater than 95% of the ganglionic neurons degenerated and the spinal cord tissues were reduced almost to monolayers. In contrast, when the recovery medium was supplemented with nerve growth factor, the ganglionic neurons and dorsal (but not ventral) cord tissue survived remarkably well. Dorsal cord neurons do not normally require an input from dorsal root ganglia for long-term maintenance in vitro, but during and after taxol exposure they become dependent for survival and recovery on the presence of neurite projections from nerve growth factor-enhanced dorsal root ganglia. (Author abstract) (37 Refs)

12. EVIDENCE OF A HEMATOPOIETIC EFFECTIVE FACTOR IN SERUM OF PATIENTS WITH POLYCYTHEMIA VERA.

Heilmann E, Holzknecht A, Fahrenkrug H
Med. Poliklinik Munster, Munster, W. Germany
Verh Dtsch Ges Inn Med; (87):772-775 1981

Possible involvement of a serum factor in polycythemia vera (PV) was studied. Hematopoietic stem cells were obtained from the peripheral blood of normal individuals. The stem cells were cultured with serum from patients with PV in the presence or absence of erythropoietin (EP). PV serum significantly increased the number of colony-forming units and blast-forming units. The increase was greater in the presence of EP than in the absence of EP. Results support the belief that a serum factor is involved in abnormal hematopoiesis in PV. (10 Refs) (In GER)

13. EFFECTS OF CONDITIONED MEDIUM FROM NON-DIFFERENTIATING OR DIFFERENTIATED MOUSE MYELOID LEUKEMIA CELLS ON FORMATION OF MACROPHAGE-GRANULOCYTE COLONIES OF NORMAL MOUSE BONE MARROW CELLS.

Okabe-Kado J, Honma Y, Hayashi M, Hozumi M
Dept. Chemotherapy, Saitama Cancer Center Res. Inst., Ina-machi, Kita-adachi-gun, Saitama-ken 362, Japan
Gan; 73(3):398-402 1982

The effects of conditioned media (CM) of various clones of mouse myeloid leukemia cells (Ml) on colony formation of normal mouse bone marrow cells were examined under various conditions. The CM of sensitive Ml cells, which could be induced to differentiate into macrophage-like and granulocyte-like cells by various factors stimulating the differentiation (D-factor), slightly inhibited colony formation of normal bone marrow cells by colony stimulating factor (GM-CSF). The CM of resistant Ml cells, which were resistant to the induction of differentiation even with high concentrations of the D-factor, significantly inhibited the colony formation of bone marrow cells by GM-CSF. The inhibitory activity of the CM from resistant cells decreased on treatment of the cells with a low concentration of actinomycin D, which could sensitize the resistant cells to induction of differentiation. The CM of differentiated sensitive Ml cells induced by dexamethasone stimulated the colony formation of bone marrow cells without exogenously added GM-CSF. Most of the GM-CSF activity in the CM was separated from the D-factor of Ml cells by Sephadex G-75 gel filtration. (Author abstract) (19 Refs)

14. GROWTH FACTOR FROM TUMOR CELLS.

Harada N, Okamoto M, Ogata Y, Shirasaka T, Fujii S
Div. Regulation of Macromolecular Function, Inst. Protein Res., Osaka Univ., Yamada-oka 3-2, Suita 565, Japan
Gan; 73(3):391-397 1982

Suitable conditions for assay of the growth factor from Yoshida sarcoma and AH-130 tumor cells were investigated using three different cell lines: baby hamster kidney cells, human embryonic lung cells and Swiss Albino mouse 3T3 embryo fibroblast cells. The results indicated that the 3T3 cell line was more sensitive to the growth stimulating activity than the other two lines. A fraction of Yoshida sarcoma tumor cells containing the growth factor showed less growth stimulating activity than a fraction of AH-130 tumor cells prepared by the same method. Using the 3T3 cell line for assay of activity, the growth factor was partially purified from AH-130 tumor cells by ammonium sulfate fractionation, DEAE-cellulose column chromatography and gel filtration on a Sephacryl S-300 column. It appeared to be thermolabile and its mol wt was estimated as about 80,000 by gel filtration on Sephacryl S-300. These findings suggest that it is a high-mol-wt substance. (Author abstract) (25 Refs)

HORMONES/ STEROIDS/ PROSTAGLANDINS AS GROWTH EFFECTORS

15. DECREASE OF TUMOR GROWTH IN MICE AFTER INTRAVENOUS THYMOSIN-TREATED BONE MARROW CELL INJECTION.

Cupissol D, Rey A, Goldstein AL, Serrou B

Laboratoire d'Immunopharmacologie des Tumeurs, INSERM U 236, ERA-CNRS No. 844, Centre Paul Lamarque, Hopital Saint-Eloi, B P 5054, 34033 Montpellier Cedex, France

JNCI; 69(1):27-31 1982

The effect of iv injection in C57BL/6 mice of 3 x 10(7) bone marrow cells preincubated in either thymosin fraction V or thymosin alpha-1 was evaluated on the growth of a 3-methylcholanthrene-induced transplantable tumor in a syngeneic system. The effect was then compared to that elicited by theophylline or levamisole, which both demonstrated thymosin-like action in vitro. The results showed significantly retarded tumor growth (P less than 0.001) and prolonged survival time (P less than 0.001) when thymosin fraction V was used. The same results were obtained with thymosin alpha-1 with use of the same protocol but only one-twentieth of the concentration of fraction V. Theophyline and levamisole demonstrated no antitumor effect. (Author abstract) (42 Refs)

16. BIOCHEMICAL RESPONSE OF LYMPHOMA CELLS TO MITOGENIC STIMULATION BY PROLACTIN.

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Mol Cell Endocrinol; 26(1/2):41-49 1982

The biochemical events involved in the mitogenic stimulation of cells by prolactin (PRL) at physiological concentrations were investigated in cultures of Nb 2 node rat lymphoma cells. After the addition of PRL (20 nanog/ml) at 18 hr to a stationary culture, cell numbers remained stable for approx 22 hr, then doubled. DNA synthesis remained low during the first 10 hr after addition of PRL, but rapidly increased to a peak at approx 17 hr. A second wave of DNA synthesis was then seen. Activity of ornithine decarboxylase (ODC) was measured in control cultures and cultures treated with PRL. Enzymatic activities of stationary cells was very low, while that of replicating cells was more than 100-fold higher. In the presence of PRL, ODC activity was substantially higher than that of cells growing in medium containing fetal calf serum. Addition of other pituitary hormones to stationary cultures had no effect on ODC activity, demonstrating that the increase in ODC activity was due to PRL per se. Addition of PRL resulted in striking increases of ODC and S-adenosyl methionine decarboxylase activities: each enzyme exhibited two peaks of activity, the second of which appeared to coincide with the increase in the rate of DNA synthesis. In the absence of PRL, cells accumulated early in the G1 phase, while PRL induced the cells to proceed through the cell cycle in a synchronized fashion. Thus, lymphoma cells are useful for studying biochemical events resulting from interaction of a mitogenic polypeptide hormone and its target cell. (24 Refs)

17. ESTROGENS STIMULATE CELL PROLIFERATION AND INDUCE SECRETORY PROTEINS IN A HUMAN BREAST CANCER CELL LINE (T47D).

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J Clin Endocrinol Metab; 55(2):276-283 1982

The effects of estradiol (E2) on two cloned sublines derived from the T47D human breast cancer cell line have been studied in vitro. Cell proliferation was evaluated by DNA assay, cell counts, and thymidine incorporation. The rate of synthesis of proteins released into the cell culture medium was assayed by [35S]methionine incorporation and polyacrylamide gel electrophoresis, followed by fluorography. In clone 11, which contains estrogen and progesterone receptors, estradiol (1 pM-1 nM) stimulated cell proliferation 2-to 5-fold after a lag period of 6 days. Maximal stimulation was observed with 1% of 3% fetal calf serum and without added insulin. The effect of E2 was biphasic, since the growth rate was stimulated for E2 concentrations less than 10 nM and then progressively inhibited for higher concentrations. Dexamethasone, dihydrotestosterone, progesterone, and R5020 (at 1 nM or 1 uM) did not modify cell growth. The antiestrogen Tamoxifen (1 uM) inhibited the E2-induced stimulation and decreased the growth of control cells. Estrogen also stimulated 2-to 3-fold the synthesis of approximately 60K mol wt dalton proteins which were released into the medium. This effect was seen as early as 1 day of E2 treatment, and a plateau of stimulation was reached with 0.1 nM E2. By contrast, in clone 8, which contains low concentrations of estrogen and progesterone receptors. E2 had no effect on cell growth and stimulated slightly the synthesis of a 55K protein. These results demonstrate that E2 is able to directly stimulate the proliferation of human epithelial breast cancer cells after having stimulated the synthesis of approximately 60,000 dalton proteins released into the medium. (Author abstract) (30 Refs)

18. CELL COMPETENCE FOR INDUCTION OF DIFFERENTIATION BY INSULIN AND OTHER COMPOUNDS IN MYELOID LEUKEMIC CLONES CONTINUOUSLY CULTURED IN SERUM-FREE MEDIUM.

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Blood; 60(1):208-212 1982

Clones of myeloid leukemic cells varying in their competence for induction of differentiation have been continuously grown in serum-free medium. In the medium used, which contained transferrin, the growth rates of these cells were nearly similar to those found in serum-containing medium. The clones also maintained in this medium their competence for induction of differentiation by the normal macrophage and granulocyte differentiation-inducing protein MGI-2, the steroid dexamethasone, and lipopolysaccharide. In contrast to the results with these inducers, some clones continuously cultured in a serum-free medium showed a gain of inducibility by insulin and another clone a gain of inducibility by the tumor promoter 12-O-tetradecanoylphorbol-13-acetate in low serum and serum-free medium. Induction of differentiation by these two compounds was therefore inhibited in these clones by the presence of serum. It is suggested that serum-free medium may also show the existence of other inducers of differentiation not detected in serum-containing medium and that these results are relevant to the possible therapeutic use of compounds such as insulin

for the induction of normal differentiation in leukemic cells in vivo. (Author abstract) (24 Refs)

19. INSULIN PROMOTES THE GROWTH OF F9 EMBRYONAL CARCINOMA CELLS APPARENTLY BY ACTING THROUGH ITS OWN RECEPTOR.

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Biochem Biophys Res Commun; 106(3):974-980 1982

Antibody directed against the insulin receptor (anti R) was used to investigate whether insulin acts through its own receptor to promote growth of F9 embryonal carcinoma cells. Proliferation of F9 cells was enhanced by the addition of anti R to the serum-free growth medium, with a max response at 30 ug/ml anti R. Thus, occupancy of the insulin receptor stimulated F9 cell growth. In the absence of mitogen, F9 cells exhibited a low level of growth; high concentrations of control IgG enhanced the basal growth of these cells. The binding properties of the F9 insulin receptor were characterized. At equilibrium 50% of the 125I-insulin tracer was effectively displaced by 10 nanog/ml of unlabeled insulin. Some competition for 125I-insulin binding to F9 cells was exhibited by greater than 500 nanog/ml of specific cell receptors for multiplication-stimulating activity (MSA) and proinsulin. With 25 ug/ml proinsulin, half max displacement of 125I-insulin occurred, while 2 ug/ml MSA produced only a 35% decrease in 125I-insulin binding. Scatchard analysis demonstrated heterogeneity or negative cooperativity between insulin receptors. Preincubation with anti R for 30 min specifically blocked 125I-insulin binding, but not binding of 125I-MSA. With 40 ug/ml anti R, 125I-insulin binding decreased 50%. Control IgG did not displace insulin tracer. Thus, anti R probably exerts its growth-promoting effect by acting through the insulin receptor. (18 Refs)

20. GROWTH RESPONSES OF RAT STOMACH CANCER CELLS TO GASTRO-ENTERO-PANCREATIC HORMONES.

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Int J Cancer; 30(1):65-67 1982

Various hormones and peptides were added to rat stomach cancer cells growing in vitro in a serum-free medium and the cell number was determined by a spectro-photometric method. Five gastro-entero-pancreatic hormones or related peptides (tetragastrin, glucagon, secretin, cholecystokinin-pancreozymin and cerulein) significantly increased the number of stomach cancer cells from 150% to 310% of the number of control cells cultivated in a serum-free, hormone-free medium. On the other hand, insulin and vasoactive intestinal peptide, and other hormones (thyroxin, epinephrine, hydrocortisone, beta-estradiol, progesterone, testosterone), peptone broth and bovine serum albumin had no significant growth effect. All the active substances belong to the two major families of gastro-entero-pancreatic polypeptide hormones, suggesting the existence of hormone receptors at the surface of stomach cancer cells. (Author abstract) (10 Refs)

21. THYROTROPIN RELEASING HORMONE RECEPTORS IN REGULATION OF PROLACTIN GENE EXPRESSION IN GH CELLS.

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Biochem Biophys Res Commun; 106(3):811-817 1982

The thyrotropin releasing hormone (TRH) receptor-modulated regulation of prolactin (PRL) gene expression was studied in three GH cell strains (rat pituitary tumor cells in culture). The three strains synthesize different amounts of PRL and exhibit different responses to TRH. Binding affinities and the number of receptors/cell were studied and the effect of epidermal growth factor on PRL gene expression in the different cell strains was explored. One strain (GH4C1) responded to TRH by stimulating PRL production while the hybrid strain (928-9b) and the bromodeoxyuridine-resistant strain (F1BGH12C1) did not. When binding of 3H-TRH was studied as a function of TRH concentration, the affinities of TRH to its specific receptors were similar in both GH4C1 and 928-9b cells. However, the number of TRH receptors/cell was significantly different in the two strains; GH4C1 cells had a max number of specific binding sites of 1.05 x 10(5)/cell, while 928-9b cells had a max number of 0.16 x 10(5)/cell. Thus, in the two strains, the TRH-responsive GH4C1 cells and the TRH-nonresponsive cells, the total number of TRH binding sites per cell was much less in the TRH-nonresponsive cell line. The inadequate number of TRH receptors in 928-9b cells may be responsible for the nonresponsive property of the hybrid cells to TRH and a possible role of the number of specific receptors in the regulation of the peptide hormone action is suggested. (11 Refs)

CYCLIC NUCLEOTIDE/ POLYAMINES/ NUCLEOTIDES AS GROWTH EFFECTORS

22. GENERAL PROCESS OF INDUCTION OF SQUAMOUS METAPLASIA BY CYCLIC ADENINE NUCLEOTIDE AND PROSTAGLANDINS: MOUSE PROSTATE GLANDS.

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Cancer Res; 42(9):3682-3687 1982

Squamous metaplasia is a common abnormality of cellular development and differentiation in the epithelia of many organs, including the prostate gland in humans. Agents known to elevate the level of intracellular cyclic adenine nucleotide have been found to direct various kinds of epithelial cells onto a pathway of epidermoid (squamous) development and a differentiation marked by keratin production. We report here that the mixture of the cyclic adenine nucleotide; N6,O2'-dibutyryladenosine cyclic 3':5'-monophosphate (0.1 mM); prostaglandins E1, E2, and B1 (each 5 ug/ml); and papaverine (1 uM) induces extensive squamous metaplasia and keratin production in the epithelial cells of mouse ventral prostate glands cultured for 3 wk. The components of the mixture act synergistically. A 10-fold higher concentration of N6,O2'dibutyryladenosine cyclic 3':5'-monophosphate (1 mM) alone is as effective as is the mixture. The presence of either the retinoid, retinylidene dimedone, or the phorbol ester, phorbol-12,13-didecanoate, at 1 uM completely prevents the induction of squamous metaplasia. The results suggest that cyclic adenine nucleotide and indirect-

ly prostaglandins E1 and E2 may play important roles in the spontaneous and exogenously induced production of squamous metaplasia. These same inducing agents have also been found in other studies to bring about squamous metaplasia and keratinization in culture in the mammary glands of mice and the breast tissue of humans, as well as to accelerate normal epidermization in chick embryo skin. The findings in prostate gland, mammary glands, and skin derived from embryonic or adult animals and humans, spanning three species and two classes of vertebrates, suggest that cyclic adenine nucleotide augmented by prostaglandins E1 and E2 may act generally on diverse types of epithelia to bring about normal and metaplastic squamous cell development and a differentiation marked by keratin production. (Author abstract) (50 Refs)

23. ANTIPROLIFERATIVE EFFECT OF (2'-5')OLIGOADENYLATE DISTINCT FROM THAT OF INTERFERON IN LYMPHOID CELLS.

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Biochem Biophys Res Commun; 107(2):511-517 1982

As previously reported, the continuously cycling lymphoma cell line P3HR-1 reacts to interferon (IFN) with growth arrest by a postmitotic accumulation in G0. A study is presented which shows that in lymphoid cells the 2'-5'oligoadenylate system does not appear to take part in this inhibition. Leukocyte IFN induces 2'-5'oligoadenylate synthetase both in PeHR-1 cells and spleen cells. Both cell types are sensitive to the antiprolifertive effects of IFN. When P3HR-1 cells were incubated in the presence of the reaction product of the synthetase, 2'-5'ApAp, rate of cell growth was reduced, and unlike IFN, the cells did not enter a plateau phase. Analysis of cell distribution showed that 2'-5'ApAp increased the number of cells in the S-phase. Appropriate controls including snake venom diesterase digested 2'5'ApAp, showed the effect was specific for the oligonucleotide trimer. No significant effect was seen after the addition of 2'5'ApA or 3'5'ApAp. IFN induced 2'5'oligo (A) synthetase in P3HR-1 cells. While IFN and 2'5'ApAp inhibited thymidine incorporation in concanavalin A-activated murine spleen cells, IFN caused a G0/G1 increase and 2'5'ApApA increased the S-phase cell number. Thus, the effect of IFN in P3HR-1 cells was not mediated by 2'5'oligo (A). (20 Refs)

24. CYTOSTATIC AND REVERSE-TRANSFORMING THERAPIES OF CANCER. A BRIEF REVIEW AND FUTURE PROSPECTS.

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Med Hypotheses; 8(6):589-612 1982

Cyclic AMP (cAMP) congeners and promoters, prostaglandin precursors, interferon, chalones, antineoplaston A, protease inhibitors, and polar solvents are reviewed. Efforts have been made to use congeners and promoters of cAMP to treat solid and ascitic animal tumors in vivo. Owing to its instability, cAMP is effective only if applied intratumoraly. The phosphodiesterase inhibitor papaverine induces reverse transformation of neuroblastoma in vitro, and was found to induce temporary remission in some clinical cases of neuroblastoma when administered in high-dose infusions. Despite inactivity in five transplantable tumor models, thioproline was taken to a clinical trial in Spain; 5 mg/kg thioproline administered po or iv produced regression of some metastatic nodules in a few patients, but only 1/17 patients (a case of renal-cell carcinoma) sustained a true partial remission. Daily ip benzaldehyde at 100 mg/kg produced 40% growth retardation of Adenocarcinoma 775 in mice, and only 10 mg/kg produced a greater than 50% retardation of Ehrlich ascites tumor. Other transplanted tumors were less responsive. No toxicity was observed or expected at these doses. Recent research indicates that retinoids exert direct cytostatic effects on many malignant cell lines in vitro, at concentrations which are not cytotoxic. Sensitivity to this effect appears to be dependent on the presence of retinoic acid-binding protein, and the in vitro efficacy of various retinoids reflects their affinity for this protein. (128 Refs)

COFACTORS/ NUTRITIONAL GROWTH FACTORS

25. VITAMIN B6 KILLS HEPATOMA CELLS IN CULTURE.

DiSorbo DM, Litwack G
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Nutr Cancer; 3(4):216-222 1982

Data are presented which indicate that vitamin B6 (pyridoxine) can retard and eventually kill Fu5-5 rat hepatoma cells in culture. Additional studies indicate that the human kidney cell line 293-31, and a rat glial cell strain, C6, display growth characteristics similar to those of hepatoma cells when cultured in 5 mM pyridoxine-supplemented medium. However, the pyridoxine-supplemented culture medium had little effect on the growth of the human mammary cancer cell line MCF-7. The resistance of the MCF-7 cells to growth inhibition by vitamin B6 suggests that the vitamer pyridoxine must be metabolized to pyridoxal before it can act as a growth inhibitor. These findings suggest the potential use of vitamin B6 as an antineoplastic agent. Possible mechanisms by which vitamin B6 promotes this effect are presented. (Author abstract) (35 Refs)

26. RETINOIDS INCREASE TRANSGLUTAMINASE ACTIVITY AND INHIBIT ORNITHINE DECARBOXYLASE ACTIVITY IN CHINESE HAMSTER OVARY CELLS AND IN MELANOMA CELLS STIMULATED TO DIFFERENTIATE.

Scott KF, Meyskens FL, Russell DH
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Proc Natl Acad Sci USA; 79(13):4093-4097 1982

Transglutaminase (TGase; R-glutaminyl-peptide:amine gamma-glutamyltransferase, EC 2.3.2.13) and ornithine decarboxylase (ODCase; L-ornithine carboxy-lyase, EC 4.1.1.17) activities were measured after the addition of retinoid analogs to Chinese hamster ovary (CHO) cells released from quiescence and Cloudman S91 (CCL 53.1) mouse melanoma cells stimulated to differentiate with alpha-melanocyte-stimulating hormone (MSH, melanotropin). In both cell culture lines, we detected a biphasic increase in TGase activity and a single peak of ODCase activity within 7 hr after release or stimulation. Retinoid analogs altered the expression of the initial TGase peak in both CHO and melanoma cells. Retinol increased the activity of TGase 1 hr after release in CHO cells, and the activity remained elevated until hr 4. A broad peak of TGase activity also occurred after the addition of alpha-difluoromethylornithne, an irreversible inhibitor of ODC-ase, and after addition of alpha-difluoromethylornithine plus retinol. In mouse melanoma cells, retinoic acid plus MSH markedly en-

hanced the activity of the initial TGase peak compared to MSH alone. Retinoic acid alone also increased TGase activity biphasically in these cells without the addition of MSH. These studies suggest that retinoid effects that increase TGase activity may alter the ODCase expression in proliferation and differentiation. (Author abstract) (36 Refs)

EXOGENOUS AGENTS AFFECTING DIFFERENTIATION

27. PHORBOL ESTERS INCREASE PROSTAGLANDIN PRODUCTION IN HUMAN OSTEOSARCOMA CELLS (MEETING ABSTRACT).

Shupnik MA, Tashjian AH
Department of Pharmacology, Harvard Medical School, Boston, MA
Toxicologist; 2(1):106 1982

Phorbol esters (PE) are potent tumor-promoting and inflammatory agents in many tissues. Twenty-[3H]-phorbol 12,13-dibutyrate (PDBu) binds to specific high affinity sites on several clonal lines of human osteosarcoma cells (G-292, TX-4, MG-63 and SaOS) with apparent Kds of 8-20 nM. Treatment with PDBu increased prostaglandin E2 (PGE2) production in 3 of the 4 cell lines; SaOS cells were unresponsive. Maximal increase in PGE2 production was 3-to 5-fold above control values, and ED50s were 10-20 mM, in agreement with the Kds for binding to the PDBu receptor. Phorbol 12-O-myristate-13-acetate (PMA) the most potent phorbol ester in terms of tumor promotion, was 6 times more potent than PDBu in increasing production of PGE2 and in competing for [3H]PDBu binding, while the biologically inactive parent alcohol phorbol had no effect on either response. The PGE2 response therefore appears to be mediated via the PDBu receptor. Ability of cells to increase PGE2 production in response to PE correlated with increased phospholipase activity which liberates arachidonate, the fatty acid precursor of PGE2. In responsive cells, PE treatment increased by 50% [3H]arachidonate release from biosynthetically labeled cell membranes. Increased lipase activity appeared to be substrate specific, since there was no increase in the release of either [14C]oleate or [3H]palmitate. All 4 cell lines, however, responded to PE as shown by decreased binding of epidermal growth factor, with ED50s of 5-15 nM. (no Refs)

28. DIRECT ACTIVATION OF CALCIUM-ACTIVATED, PHOSPHOLIPID-DEPENDENT PROTEIN KINASE BY TUMOR-PROMOTING PHORBOL ESTERS.

Castagna M, Takai Y, Kaibuchi K, Sano K, Kikkawa U, Nishizuka Y (c/o Nishizuka) Dept. Biochemistry, Kobe Univ. Sch. Medicine, Kobe 650, Japan
J Biol Chem; 257(13):7847-7851 1982

Tumor-promoting phorbol esters such as 12-O-tetradecanoylphorbol-13-acetate (TPA) directly activate in vitro Ca++-activated, phospholipid-dependent protein kinase (protein kinase C), which normally requires unsaturated diacylglycerol. Kinetic analysis indicates that TPA can substitute for diacylglycerol and greatly increases the affinity of the enzyme for Ca++ as well as for phospholipid. Under physiological conditions, the activation of this enzyme appears to be linked to the receptor-mediated phosphatidylinositol breakdown which may be provoked by a wide variety of extracellular messengers, eventually leading to the activation of specific cellular functions or proliferation. Using human platelets as a model system, TPA is shown to enhance the protein kinase C-specific phosphorylation associated with the release reaction in the total absence of phosphatidylinositol breakdown. Various phorbol derivatives which have been shown to be active in tumor promotion are also capable of activating this protein kinase in vitro systems. (Author abstract) (39 Refs)

29. EFFECTS OF 12-O-TETRADECANOYLPHORBOL-13-ACETATE ON FIBROBLASTS FROM INDIVIDUALS GENETICALLY PREDISPOSED TO CANCER.

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Cancer Res; 42(9):3870-3879 1982

The purpose of the present study was to determine whether skin fibroblasts from individuals, either with an inherited predisposition to cancer or with genetic disorders usually associated with a high risk of cancer, can be oncogenically transformed in vitro by a tumor promoter alone. The effects of chronic and limited applications of 12-O-tetradecanoylphorbol-13-acetate (TPA) on several properties that are associated with transformation were examined using skin fibroblasts from individuals with polyposis coli, a familial cancer syndrome, xeroderma pigmentosum, Fanconi's anemia, and trisomy 21. The results of this study show that TPA treatment induces similar changes on cellular morphology, growth rate, saturation density, epidermal growth factor binding, and cytoskeleton in fibroblasts from both normal and genetically predisposed individuals. None of these cell lines, however, acquired anchorage-independent growth or unlimited growth potential in culture after chronic application of TPA. These observations suggest clearly that skin fibroblasts from individuals with either a genetic predisposition to or a high risk of cancer may not exist in a preneoplastic or 'initiated' state susceptible to oncogenic transformation by TPA alone and that the mechanism of genetically determined cancer induction may be different from that of chemical carcinogenesis. (Author abstract) (28 Refs)

30. PHORBOL ESTER-INDUCED DIFFERENTIATION OF HUMAN T-LYMPHOBLASTIC CELL LINE HPB-ALL.

Nakao Y, Matsuda S, Fujita T, Watanabe S, Morikawa S, Saita T, Ito Y
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Cancer Res; 42(9):3843-3850 1982

The compound 12-O-tetradecanoylphorbol-13-acetate (TPA), a potent tumor promoter, induced phenotypic differentiation in the human thymic acute lymphocytic leukemia cell line, HPB-ALL. Within 30 min of seeding in the presence of TPA, the cells formed a smooth round shape. After a 7-day exposure to TPA, most of the cells became smaller and reminiscent of large or atypical lymphocytes. Electron microscopic analysis evidenced morphological differentiation in TPA-treated HPB-ALL cells. Thymic antigens stained with monoclonal antibody OKT6 were dramatically reduced while Leu2a-positive cells were increased in the TPA-treated HPB-ALL cells. However, OKT3-positive cells did not appear in these TPA-treated cells for up to 7 days. Upon TPA-induced phenotypic differentiation, the growth rate of cells was significantly inhibited, their ability to incorporate DNA and RNA via 3H-labeled precursors was reduced, their ability to bind sheep red blood cell rosettes was significantly increased, and the proportion of terminal deoxynucleotidyl transferase-positive cells was decreased. (Author abstract) (18 Refs)

31. A PHORBOL ESTER TUMOR PROMOTER INDUCES CHANGES IN THE EXPRESSION OF IMMUNOGLOBULINS AND DR ANTIGENS IN HUMAN LYMPHOBLASTOID CELLS.

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J Immunol; 129(2):539-544 1982

The present study indicates that 12-O-tetradecanoyl phorbol 13-acetate (TPA) can induce either increases or decreases in the relative expression of membrane-associated IgM and IgD as well as the expression of other markers of lymphocyte differentiation on human B lymphoblasts, depending on the clone studied. The changes in membrane Ig expression are apparent within 24 hr and are maximal at about 48 hr after the addition of the compound. In clone BL, TPA decreases the membrane IgM/IgD ratio as determined by the percentage of cells bearing these isotypes and by their densities on single cells. In clone MW-E, TPA has the opposite effect. Although these changes are followed by an increase in Ig secretion by MW-E cells, measured by immunofluorescence and the protein A plaque assay, There is no induction of Ig secretion in BL cells. In addition, TPA increases the expression in BL of an additional B lymphocyte marker, the D region-related (DR) antigens, while having the opposite effect in MW-E. In two sublines of Daudi, a membrane IgM-positive, IgD-negative, nonsecreting cell line, TPA treatment resulted in the expression of membrane IgD without the induction of Ig secretion. Thus, the TPA-induced changes in the IgM/IgD ratio and Dr antigen expression are inversely related and are not due to differential proliferation of subpopulations. Taken together, it appears that, depending on the particular clone of B lymphoblasts studied, TPA can induce or inhibit a coherent program of B lymphocyte maturation. (Author abstract) (52 Refs)

32. INHIBITION OF DNA REPLICATION AND REPAIR BY ANTHRALIN OR DANTHRON IN CULTURED HUMAN CELLS.

Clark JM, Hanawalt PC
Dept. Biological Sciences, Stanford Univ., Stanford, CA, 94305
J Invest Dermatol; 79(1):18-22 1982

The comparative effects of the tumor promoter anthralin and its analog, danthron, on semiconservative DNA replication and DNA repair synthesis were studied in cultured human cells. Bromodeoxyuridine was used as density label together with 3H-thymidine to distinguish replication from repair synthesis in isopycnic CsCl gradients. Anthralin at 1.1 uM inhibited replication in T98G cells by 50%. In cells treated with 0.4 or 1.3 uM anthralin an additive effect was observed on the inhibition of replication by ultraviolet light (254 nm). In cells irradiated with 20 J/m2, 2.3 uM anthralin was required to inhibit repair synthesis by 50%. Thus there was no selective inhibitory effect of anthralin on repair synthesis. Danthron exhibited no detectable effect on either semiconservative replication or repair synthesis at concentrations below about 5.0 uM. Neither compound stimulated repair synthesis in the absence of ultraviolet irradiation. Thus, anthralin and danthron do not appear to react with DNA to form adducts that are subject to excision repair. Although both compounds appear to intercalate into supercoiled DNA in vitro to a limited extent, the degree of unwinding introduced by the respective drugs does not correlate with their relative effects on DNA synthesis in vivo. Therefore the inhibitory effect of anthralin on DNA replication and repair synthesis in T98G cells does not appear to result from the direct interaction of the drug with DNA. (Author abstract) (20 Refs)

33. FAILURE OF PHORBOL ESTER TPA TO PROMOTE GROWTH OF IN VIVO-INITIATED RAT HEPATOCYTES TRANSFERRED INTO A CULTURE SYSTEM.

Kayano T, Nomura K, Hino O, Kitagawa T
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Gan; 73(3):354-357 1982

The effect of 12-O-tetradecanoylphorbol-13-acetate (TPA), a potent promoter in epidermal carcinogenesis, on the growth of rat hepatocytes initiated in vivo and transferred into a culture system was investigated. The hepatocytes were isolated by enzymatic perfusion from the rat liver after 12 wk of acetylaminofluorene feeding or 2 to approx 6 wk of diethylnitrosamine treatment. TPA did not show any promotive effect in terms of the number and size of proliferative epithelial cell foci derived from initiated hepatocytes, in contrast to the remarkable effect of phenobarbital, which is a potent promoter in hepatocarcinogenesis. The results suggest the existence of organ or target cell specificity of promoters in the two-stage concept of carcinogenesis. (Author abstract) (16 Refs)

34. CLONAL AND BIOCHEMICAL ANALYSIS OF COMMITMENT OF HL-60 CELLS TO TERMINAL DIFFERENTIATION (MEETING ABSTRACT).

Tsiftsoglou AS, Wong W, Minden M, Robinson SH
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Conference on Cell Proliferation, Cancer and Cancer Therapy, February 17-19, 1982, New York, New York New York Academy of Sciences; :AP-23 1982

We have used a modified cloning assay to study commitment of human promyelocytic leukemia (HL-60) cells to terminal differentiation. This system employs the standard plasma clot reagents plus L-glutamine. Cells were treated with DMSO (1.5%), retinoic acid (RA) (2uM), or no drug, and at various times removed from culture and plated in plasma clots. Parallel biochemical and morphological studies were carried out in primary culture to determine the degree of HL-60 maturation. The major findings are summarized as follows: (a) HL-60 cells can be cloned in plasma clots with a plating efficiency of 40-60%; (b) Cells exposed to DMSO and RA form colonies composed either of all NBT (Nitro Blue Tetrazolium) positive cells or both NBT+ and NBT- cells in plasma clots; With increasing time of exposure to DMSO or RA both colony number and size decline progressively until colony formation finally ceases to occur. Undifferentiated cells give rise to compact colonies without much cell migration; (c) Commitment of HL-60 cells to terminal maturation and loss of proliferative capacity is irreversible; and (d) HL-60 cell differentiation induced by RA or DMSO is associated with a decline in RNA and DNA synthesis. (1 Ref)

35. RESPONSES OF PRENEOPLASTIC EPIDERMAL CELLS TO TUMOR PROMOTERS AND GROWTH FACTORS: USE OF PROMOTER-RESISTANT VARIANTS FOR MECHANISM STUDIES.

Colburn NH, Wendel E, Srinivas L
Lab. Viral Carcinogenesis, NCI, Frederick, MD, 21701
J Cell Biochem; 18(3):261-270 1982

The mechanism of late stage promotion was studied in the JB6 mouse epidermal cell line. Second-stage promoters, including phorbol diesters which act both as first- and second-stage promoters, and mezerein, which acts primarily as a second-stage promoter, showed activity in promotion of transforma-

tion in JB6 cells. The second-stage antipromoter retinoic acid inhibited promotion of transformation by 12-O-tetradecanoyl-phorbol-13-acetate (TPA) but the antiproteases antipain and leupeptin, which inhibit first-stage promotion, did not, even at concentrations greater or equal to those that inhibited promotion of transformation in 10T1/2 cells. Three of four mitogen-resistant lines, R219, R6141, and R23, were promotable to anchorage independence by TPA. Thus, promotion of transformation by TPA in JB6 cells can occur without the release-from-quiescence of mitogenic response to TPA. Cross-resistance to mezerein and epidermal growth factor (EGF) occurred in two TPA promotion-resistant clones of JB6, C130, and C125. C125 and C130 showed moderate to high levels of EGF binding while three promotable lines, R219, R6141, and R23, showed little or no EGF binding. Thus, EGF binding is not a requirement for promotion of transformation in JB6 cells by TPA. The role of TPA-induced changes in the sialic-acid-containing plasma membrane glycolipids was investigated. TPA produced a 10-fold decrease in precursor incorporation into a trisialoganglioside and a 2-fold increase in incorporation into an unidentified ganglioside. These findings suggest that gangliosides may be important in promotion of tumor cell phenotype. A model for the sequence of events necessary for promotion of transformation in JB6 cells is proposed. (32 Refs)

36. DIMETHYLSULFOXIDE-INDUCED ALTERATIONS IN THE GROWTH PROPERTIES AND PROTEIN COMPOSITION OF IN VITRO-PROPAGATED MURINE HEPATOMA CELLS.

Higgins PJ, O'Donnell PV
Lab. Investigative Cytology, Section 6147, Memorial Sloan-Kettering Cancer Center, 1275 York Ave., New York, NY, 10021
Oncology; 39(5):325-330 1982

The effects of the differentiation-inducing agent dimethylsulfoxide (DMSO) on the growth properties and protein synthetic capacity of BW77-1 mouse hepatoma cells were compared at various media concentrations of the polar solvent. DMSO produced a dose-dependent reduction in final population density, reduced or eliminated cell piling and stimulated synthesis of albumin. The rising albumin content reflected dose-related DMSO-induced increases in total cellular protein and in the albumin contribution to total cellular protein. In order to determine whether viral gene expression was associated with DMSO-induced stimulation of albumin synthesis, BW77-1 cultures were examined for the production of ecotropic and xenotropic Type C virus. The BW77-1 hepatic tumor cell line was determined to be a nonproducer of Type C virus by assays designed to measure extracellular reverse transcriptase, viral env gene product and infectivity on mouse and mink indicator cells. Type C virus could not be induced in BW77-1 cultures by treatment with DMSO under conditions which lead to a reduced proliferative capacity and enhanced expression of liver-specific genes. (Author abstract) (25 Refs)

37. EXPRESSION OF NADH-CYTOCHROME B5 REDUCTASE DURING DIMETHYL SULFOXIDE-INDUCED DIFFERENTIATION OF FRIEND ERYTHROLEUKEMIA CELLS.

Lostanlen D, Kaplan JC
Institut de Pathologie Moleculaire, Groupe INSERM U129, 24 rue du Faubourg Saint-Jacques, 75014 Paris, France
FEBS Lett; 143(1):35-39 1982

The activity and distribution of membrane-bound and soluble NADH-cytochrome b5 reductase (Cb5R) before and after induction by dimethyl sulfoxide (DMSO) was studied in Friend murine erythroleukemic cells (MELC). Exposure of cells to 1.5% DMSO for 4 days resulted in approx 2-fold increase in specific activity of Cb5R (3-fold when expressed per 10(8) cells). A difference in enzyme specific activity (+21%) was detected after 2 days during active proliferation; the increase became more pronounced (+76%) after 4 days, when the cells entered stationary phase. The kinetics of the increase in enzyme activity follow the kinetics of Hb production, and show a good correlation between the two processes. Specific Cb5R activities in a DMSO-resistant variant, non-inducible for Hb and spectrin, were increased 12%, 18%, and 24%, respectively, after 2, 4, and 6 days of DMSO treatment, Enzyme activities increased markedly after 2, 4, and 6 days of DMSO treatment in the 12,000 x g pellet and two membranous fractions, but not in the cytosolic fraction. Reticulocytes and erythrocytes of DBA/2 mice from which the MELC originated differed in relative amounts of soluble and membrane-bound enzyme. The soluble and membrane-bound forms in reticulocytes were 43% and 57%, respectively; the same quantities in erythrocytes were 69% and 31%. In immature erythroid cells Cb5R is apparently membrane-bound, and can be induced by DMSO treatment. The enzyme is synthesized as a membrane-bound form during the proliferative phase of erythroid differentiation and the soluble form is released by proteolysis at a late stage of differentiation. (22 Refs)

38. TUMOR PROMOTER TELEOCIDIN INHIBITS INTERNALIZATION AND NUCLEAR ACCUMULATION OF EPIDERMAL GROWTH FACTOR IN CULTURED HUMAN HEPATOMA CELLS.

Kaneko Y
First Dept. Medicine, Univ. Tokyo, 7-3-1, Hongo, Bunkyo-ku, Tokyo 113, Japan
FEBS Lett; 143(1):5-8 1982

The effects of teleocidin, 12-O-tetradecanoyl-phorbol-13-acetate (TPA), and retinoic acid on mitogenesis and epidermal growth factor (EGF) binding were characterized in human HUH hepatoma cells. Teleocidin (10 nanog (n)g/ml) inhibited the proliferation of cells in response to both fetal calf serum (FCS; 10%) and EGF (50 ng/ml) in low-serum medium. TPA and retinoic acid also inhibited FCS-induced proliferation. Incorporation of [3H]leucine by cells grown in the presence of teleocidin was virtually identical to that by untreated cells, indicating that teleocidin did not interfere with protein synthesis. Teleocidin and TPA, but not retinoic acid, reduced the amount of surface-bound 125I-EGF during the first 3 hr, but little change in surface-bound EGF was observed after 6 hr. The effect of teleocidin on EGF internalization depended upon the concentration of the drug; it was half-maximal at 0.3 ng/ml and virtually maximal at 10 ng/ml. At all teleocidin concentrations, the amount of surface-bound and internalized EGF was slightly greater in the presence of retinoic acid. The ability of teleocidin to inhibit EGF internalization may result from structural and/or functional alterations of the cell membrane. (22 Refs)

39. EFFECTS OF PEROXIDES ON RODENT SKIN: EPIDERMAL HYPERPLASIA AND TUMOR PROMOTION.

Klein-Szanto AJ, Slaga TJ
Biology Div., Oak Ridge Natl. Lab., P.O. Box Y, Oak Ridge, TN, 37830
J Invest Dermatol; 79(1):30-34 1982

Free radical generating peroxides are potent skin irritants. After a single topical application of either 10, 20, or 40 mg of lauroyl peroxide or benzoyl peroxide on the dorsal skin of Sencar mice, the epidermal thickness increased markedly. No major inflammatory or vascular alterations were noted. On the other hand, 15 or 30% hydrogen peroxide produced an extensive epidermolysis, as well as inflammation and vascular injury, followed by quick regeneration and epidermal hyperplasia. Both lauroyl peroxide- and benzoyl peroxide-induced hyperplasias were characterized by a sustained production of dark basal keratinocytes, which constituted approx 10% of the basal cell population during the 1st wk after single topical application. Hydrogen peroxide-induced epidermal hyperplasias also exhibited numerous dark cells, but their presence was less sustained. Although all these peroxides were inactive either as initiators or as complete carcinogens, lauroyl peroxide was as effective as benzoyl peroxide when used as a skin tumor promoter in a two-stage carcinogenesis protocol. In a similar experimental protocol, hydrogen peroxide proved to be a very weak skin tumor promoter. (Author abstract) (16 Refs)

OTHER RELATED STUDIES

40. GLIAL DIFFERENTIATION IN MEDULLOBLASTOMA: HISTOGENETIC INSIGHT, GLIAL REACTION, OR INVASION OF BRAIN (MEETING ABSTRACT).

Coffin CM, Mukai K, Dehner LP
Department of Laboratory Medicine and Pathology, University of Minnesota, Minneapolis, MN
Lab Invest; 46(1):14A 1982

Recent studies applying the immunohistochemical demonstration of glial fibrillary acidic protein (GFAP) specific for astroglia and ependyma have described a high frequency (84 per cent) of glial differentiation. Twenty cases of medulloblastoma (21 months to 46 years old 10 males and 10 females) were examined histopathologically in order to assess the presence of brain matter in the sections and/or fibrillary background in the tumor. Sections from approximately identical areas were also stained for GFAP, as well as metastatic deposits of medulloblastoma in bone marrow or other extracranial sites in four cases. The principal, although not exculsive, staining reaction for GFAP was located at the interface with normal brain, the periphery of tumor nests, or in perivascular sites. None of the metastatic lesions demonstrated GFAP. Our observations have generated a note of reservation in the interpretation of previous studies describing a high incidence of glial differentiation in medulloblastoma. Three cases in our study had histologic features which were very similar to conventional neuroblastoma including the presence of ganglion cells in two neoplasms in the absence of tumor elsewhere. Mature glial elements were demonstrated electron microscopically in two cases. In one of these, GFAP positivity was present in the primary cerebellar tumor but was absent in a subsequent bone marrow metastasis. (1 Ref)

41. THE ROLE OF DIFFERENTIATION IN THE SUPPRESSION OF TUMORIGENICITY IN HUMAN CELL HYBRIDS.

Peehl DM, Stanbridge EJ
(c/o Stanbridge) Dept. Microbiology, California Coll. Medicine, Univ. California, Irvine, CA, 92717
Int J Cancer; 30(1):113-120 1982

HeLa human keratinocyte hybrid cell populations behave like transformed cells in culture but do not form progressive tumors when inoculated into athymic nude mice. In this respect they behave like HeLa/fibroblast hybrids. However, the small nodules that do form occasionally have the appearance of moderate to highly differentiated squamous-cell carcinomas. These observations suggest that differentiation might be the signal suppressing growth of human hybrid cells in vivo. Differentiation occurs rapidly and is not site-specific. Reconstitution of HeLa/keratinocyte hybrid cells in culture from differentiated nodules gave rise to a line of stem cells, capable of renewed proliferation and differentiation, and a line which lost the ability to differentiate and formed large, progressive tumors when reinjected. These tumors are anaplastic carcinomas that resemble tumors formed both by HeLa and tumorigenic segregant HeLa/fibroblast hybrid cells. Morphological evidence indicates that non-tumorigenic HeLa/fibroblast hybrid cells also respond to differentiation signals, whereas the tumorigenic segregants do not. Furthermore, the non-tumorigenic hybrid cells in the nude mouse take on the 'phenotypic signature' of the normal parental cell irrespective of the tumorigenic parental cell. Efforts to identify factors in vitro mimicking these differentiation signals have so far failed, but such factors may be important elements in the regulation of proliferation in vitro. (Author abstract) (40 Refs)

42. REGULATORY MECHANISMS IN MAMMALIAN EMBRYOGENESIS: CONTROL OF EXPRESSION OF SIMIAN VIRUS 40, HISTOCOMPATIBILITY AND BETA-2 MICROGLOBULIN GENES IN DNA- TRANSFORMED MURINE TERATOCARCINOMA CELLS.

Linnenbach AJ
University of Pennsylvania, Philadelphia, PA
Diss Abstr Int [B]; 42(6):2229-B 1981

Teratocarcinoma cells, the malignant, pluripotent stem cell of murine teratomas, have proved to be a fruitful model for the study of differentiation. We have constructed by transformation the thymidine kinase deficient F9 cell, with a selectable pBR322/HSV-1 tk/SV40 recombinant DNA, an F9 teratocarcinoma stem cell line, 12-1, which stably carries one copy per cell of the tripartite plasmid in a chromosomal site. Like its parental teratocarcinoma stem cell, the 12-1 line (1) expresses stage specific embryonic antigen-1 (SSEA-1) (2) does not express H-2 or beta-2 microglobulin, (3) does not express SV40 T-antigen, and (4) has retained the ability to differentiate in the presence of retinoic acid. The differentiated cells no longer express SSEA-1 while acquiring the H-2, beta-2 and SV40 T-antigens. The organization of the SV40 genome and its expression at the RNA and protein levels has been compared in 12-1 stem cells and a differentiated daughter cell, 12-1a, and the analysis indicates that while SV40 mRNA species are present and apparently processed identically in the two cell types, SV40 early proteins are not detected in stem cells by immunofluorescence or immunoprecipitation, indicating that regulation of expression of these proteins in stem cells is a post-transcriptional event. The differential expression of H-2 antigen, and beta-2 microglobulin in the same stem and differentiated cells

is, however, due to a lack of transcription of these genes in stem cells. Thus, expression of specific genes during differentiation in murine teratocarcinoma cells, and by analogy in early mouse embryos, can be regulated by at least two different mechanisms. (no Refs)

43. POLYOMA VIRUS MUTANTS AS PROBES OF VARIETY AMONG MOUSE EMBRYONAL CARCINOMA CELL LINES.

Georges E, Vasseur M, Blangy D
(c/o Blangy) Laboratoire des Virus Oncogenes, Universite Pierre et Marie Curie, IRSC, B.P. No 8, F-94802 Villejuif Cedex, France
Differentiation; 22(1):62-65 1982

Embryonal carcinoma (EC) cells are resistant to infection with polyoma virus and become permissive when allowed to differentiate. Polyoma host-range (PyEC) mutants have been selected on two embryonal carcinoma cell lines, PCC4 and F9, which differ in their differentiation potential, both in vitro and in vivo. Since PyEC mutants selected on one line failed to develop, or developed only poorly on the other, we used these two classes of mutants as probes towards several EC lines which differed in their origin and differentiation properties. From their susceptibility to either mutant, and from the effect of temperature upon the efficiency of infection, we inferred a classification of these teratocarcinoma cell lines, which is in agreement with a previous one based upon metabolic coupling. We also discuss results indicating that different EC cell lines might represent steps in the process of cell determination at the embryonic level. (Author abstract) (26 Refs)

44. GROWTH OF HUMAN HEPATOMA CELL LINES WITH DIFFERENTIATED FUNCTIONS IN CHEMICALLY DEFINED MEDIUM.

Nakabayashi H, Taketa K, Miyano K, Yamane T, Sato J
(c/o Sato) Div. Pathology, Cancer Inst., Okayama Univ. Sch. Medicine, 2-5-1 Shikata-cho, Okayama 700, Japan
Cancer Res; 42(9):3858-3863 1982

A human hepatoma cell line, HuH-7, which was established from a hepatocellular carcinoma, was found to replicate continuously in a chemically defined medium when the medium was supplemented with Na2SeO3. The cells grew better in this medium than in serum-containing medium without any adaptation period. Other established human hepatoma and hepatoblastoma cell lines, HuH-6 cl-5, PLC/PRF/5, huH-1, and huH-4, also grew in the defined medium. Although HLEC-1 cells failed to proliferate continuously with Na2SeO3 alone, they grew if a cell-free conditioned medium from HuH-7 cells was added to the medium. These cell lines, except the HLEC-1 cell line, produced the following human plasma proteins among those examined: albumin, prealbumin, alpha1-antitrypsin, ceruloplasmin, fibrinogen, fibronectin, heptoglobin, hemopexin, beta-lipoprotein, alpha2-macroglobulin, beta2-microglobulin, transferrin, Complement Components 3 and 4, and alpha1-fetoprotein. Beside plasma proteins, the media from HuH-7, HuH-6 cl-5, PLC/PRF/5, and huH-1 contained anti-carcinoembryonic antigen-reactive proteins, and those from PLC/PRF/5, huH-1, and huH-4 medium contained hepatitis B surface antigen. These proteins were detected during periods of serial cultivation over 9 mo under the above culture conditions. The hepatoma cell lines grown in the fully defined synthetic medium may provide a new approach for investigating the growth and metabolism of human hepatoma cells in vitro. (Author abstract) (35 Refs)

45. SEPARATION AND CHARACTERIZATION OF NEOPLASTIC CELL SUBPOPULATIONS OF A TRANSPLANTABLE RAT PANCREATIC ACINAR CARCINOMA.

Becich MJ, Reddy JK
(c/o Reddy) Dept. Pathology, Northwestern Univ. Medical Sch., 303 East Chicago Ave., Chicago, IL, 60611
Cancer Res; 42(9):3729-3740 1982

The transplantable pancreatic acinar carcinoma established in F344 rats demonstrates heterogeneity of cytodifferentiation with cell types ranging from those containing no zymogen granules to cells with mature zymogen differentiation. Two relatively homogeneous subpopulations of cells were isolated by isopyknic Percoll gradient centrifugation from the heterogeneous cell population of this tumor. The subpopulation obtained at a density of 1.0987 g/ml was designated the granule-enriched fraction (GEF) and contained morphologically differentiated cells with abundant mature zymogen granules. The other subpopulation, obtained at a density of 1.0789 g/ml, consisted of poorly differentiated cells lacking zymogen maturation and was thus termed the granule-deficient fraction (GDF). In both fractions, greater than 97% of the cells were viable and maintained linear rates of [3H]leucine and [3H]thymidine incorporation into protein and DNA for up to 3 hr following isolation. Twenty percent of the cells in GDF subpopulation and 12% of the cells in GEF subpopulation synthesize DNA. Distinct differences in nuclear:cytoplasmic ratio, secretory granule content, and degree of polarity were found between GDF and GEF. The GEF subpopulation contained high levels of amylase and lipase, while the GDF subpopulation displayed very low levels of these enzymes. Both subpopulations produced tumors when injected sc into F344 rats. Concanavalin A (Con A) receptors were found on pancreatic carcinoma cells regardless of the extent of cytodifferentiation in contrast to normal pancreatic embryogenesis, in which Con A receptors are discerned only in acinar cells containing mature secretory granules. This finding suggests either that the neoplastic transformation results in altered genetic expression or that pancreatic carcinoma arises from dedifferentiation of mature acinar cells which normally possess Con A receptors. (52 Refs)

46. ROLE OF ENZYMATIC METHYLATION OF DNA IN CELL DIFFERENTIATION AND TRANSFORMATION.

Fedorov NA
Central Res. Inst. Hematology and Blood Transfusion, Moscow, USSR
Eksp Onkol; 4(3):3-11 1982

Literature data on DNA methylation are reviewed, with special emphasis on the roles of specific DNA methylases and S-adenosylmethionine (SAM) in cell differentiation and neoplastic transformation. Determinations of 5-methylcytosine contents of leukemic and normal lymphocytes showed a marked elevation of DNA methylation in the leukemic cells. Changes in rate and specificity of DNA methylation were observed during chemical carcinogenesis and following administration of antineoplastic agents. A number of SAM analogs were shown to inhibit cell proliferation. (73 Refs) (In RUS)

47. SERUM-FREE GROWTH OF NORMAL AND TUMOR MOUSE MAMMARY EPITHELIAL CELLS IN PRIMARY CULTURE.

Imagawa W, Tomooka Y, Nandi S
Cancer Res. Lab., Univ. California, Berkeley, CA, 94720
Proc Natl Acad Sci USA; 79(13):4074-4077 1982

Freshly isolated normal and tumor mouse mammary epithelial cells embedded within a collagen gel matrix undergo sustained growth when cultured for as long as 3 wk in a serum-free medium composed of a 1:1 (vol/vol) mixture of Hepes-buffered Ham's F12 and Dulbecco's modified Eagle's medium supplemented with insulin, epidermal growth factor (EGF), transferrin, bovine serum albumin fraction V, and cholera toxin. Of these additives, only insulin, EGF, and albumin are required for the growth of most normal cells. Albumin is not always an absolute requirement for growth but greatly enhances it. Lithium has been found to stimulate the growth of normal cells and can replace EGF. The collagen matrix culture system allows sustained growth of primary cultures of both normal and neoplastic mammary epithelium in serum-free conditions. This serum-free system will be useful in identifying and investigating the role of hormones, growth factors, and nutritional factors in regulating the growth of mammary epithelial cells. (Author abstract) (35 Refs)

48. REPLICATION OF PLURIPOTENT STEM CELLS IN GRANULOCYTE/MACROPHAGE COLONIES DEVELOPED IN METHYLCELLULOSE CULTURE OF MOUSE BONE MARROW CELLS.

Miyanomae T, Tsurusawa M, Mori KJ, Mikawa H, Ito Y
Dept. Microbiology, Pediatrics, Kyoto Univ., Sakyo-ku, Kyoto 606, Japan
Gan; 73(3):408-412 1982

The existence of pluripotent hemopoietic stem cells in granulocyte/macrophage colonies was demonstrated in a culture of mouse bone marrow cells in semi-solid methylcellulose with spleen conditioned medium as colony-stimulating activity. When fresh growth medium containing the conditioned medium was supplemented to 1-wk culture, the number of cells per colony increased significantly in the next wk. The number of pluripotent stem cells also increased by a factor of 12 or more in the 2nd wk of the culture. The number of cell divisions in such a culture was calculated as 12-15 in 2 wk. These results indicate that some of the granulocyte/macrophage colonies are derived from pluripotent stem cells, and that the stem cells can undergo three to five self-replications in such semi-solid culture. (Author abstract) (16 Refs)

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